

**“Development of self-adhesive, remineralising and
antibacterial composites and the effect of these properties on
the bond strength, monomer conversion and resin tag
formation in human dentine”**

Submitted by

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Declaration of originality

I declare that the work represented in this thesis is the result of my own investigations, except where otherwise stated. Information from the published and unpublished work of others had been acknowledged in the text and the relevant references are included in this thesis.

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Signature: ----- Date: -----

Dedication

To my Mother

This thesis is dedicated to my mother whose care and endless love and support throughout my life have been the reason behind every success from the smallest to the greatest.

She raised me to love education; she taught me that freedom and independence, among the most important things in life, could be pursued through education and she always advised me to work with dedication and ethics. She has unconditionally been by my side in every decision I have taken in my life, right or wrong.

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Abstract

Dental composite materials have been used for over 50 years for the restoration of a tooth cavity and nowadays they largely replace the use of amalgam. Current dental composites though, have multiple weaknesses that can lead to failure of the restorations such as recurrent caries and multiple step placement.

Aims: The aim of this project was to develop new novel composite formulations that promote remineralising, antibacterial and self-adhesive properties, which could potentially improve the above-mentioned disadvantages of the dental composites, to assess the effect of the new components on the bond strength. It also aimed to assess if the final formulation SU2, has sufficient monomer conversion for single step bulk placement and can form resin tags into carious versus affected dentine.

Materials and methods: Three experimental formulations (A6, C2, SU2) with powder part containing different amounts of MCPM and polylysine and monomer consisting of UDMA, PPGDMA, 4META and CQ. The bond strength of two new formulations was assessed and compared to commercial Z250 and Fuji II & IX glass ionomer and monomer conversions (MC) at depths of 2 mm with 20 s versus 4 mm with 40 s light exposure were determined using ATR FTIR for one experimental formulation. A commercial composite (Z250, 3M) and a resin modified glass ionomer cement (Fuji II-LC, GC) were used as controls (n=3). Sound adult molars and premolars were collected and embedded into self-curing resin blocks to assess the shear bond strength in human dentine.

Results: The shear bond strength of C2 found to be higher compared to A6 when no etch or i-bond were used. The commercial Z250 had the lowest bond strength without etch and bond, but was still best performing when etch and bond were used together, prior to the application of the material. For the SU2 formulation 75% and 70% monomer conversion was obtained at 2mm and 4mm depth respectively. For Z250, results were 56% and 53% and for Fuji II-LC 90% and 93% respectively. For the experimental but not commercial material, resin tag formation up to 200 micron in length was observed in both highly infected dentine and the underlying disease affected dentine by the various microscopic techniques.

Impact Statement

Dental caries is one of the most prevalent diseases worldwide and affects many individuals including young children. It can have an impact on many aspects of everyday life and on the quality of life as it can cause major pain and discomfort. School attendance and performance can be affected if there is pain from dental caries in young children. Untreated dental caries can lead to tooth loss. As a result it can affect not only the function but also the aesthetics of the face, leading to a psychological distress of the affected individuals and an overall negative result on social life. In deprived areas especially, where the access to care is limited, dental caries can be a significant health burden.

Many materials have been used over the years for the restoration of caries cavities; all of them however have some drawbacks associated either with their strength and longevity, or with their level of aesthetics. This project is proposing a new material that can combine all the ideal properties that a modern restorative material should possess.

First of all the experimental formulations have been shown to have self-adhesive properties and shear bond strength that is better compared to commercial composites without the prior etching/ bonding of the dentine. As a result, they can reduce the amount of time and the level of technique sensitivity, making the application easier and quicker for both the dentist and the patient. They also have remineralising and antibacterial properties meaning that they can potentially remineralise remaining caries below a restoration, something that would be a great privilege especially when it comes to paediatric patients that the caries removal is more challenging. The degree of monomer conversion has been found to be better than that of a commercial composite and finally, previous research on these formulations has shown good aesthetics and colour stability.

These new materials are promising for restoring easily and effectively caries cavities even in the presence of non-optimal moisture control, having the potential to benefit not only the field of paediatric dentistry worldwide, but also the areas with compromised access to care, such as developing countries. With a less technique-sensitive application and remineralising potential this material could be a great benefit for restoring teeth quicker and in a less than ideal situation in countries with high caries prevalence and limited access to dental treatment.

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List of abbreviations

ACP	Amorphus Calcium Phosphate
AS	Artificial Saliva
BisGMA	Bisphenol A-glycidyl methacrylate
CQ	Camphorquinone
DMAEMA	2-Dimethyl amino ethyl methacrylate
DMPT	N,N- dimethyl- p- toluidine
ECC	Early Childhood Caries
FDA	Food and Drug Administration
FDI	World Dental Federation
FTIR	Fourier Transform Infrared Spectroscopy
GIC	Glass Ionomer Cement
HA	Hydroxyapatite
HEMA	2-Hydroxyethylmethacrylate
MCPM	Monocalcium calcium phosphate monohydrate
MID	Minimally Invasive Dentistry
NTGGMA	N p-tolyl glycidyl methacrylate
PPGDMA	Poly-Propylene Glycol Dimethacrylate
PLR	Powder liquid mass ratio
PLS	ϵ -Poly-L-lysine
RMGIC	Resin Modified Glass Ionomer Cements
SBS	Shear Bond Strength
SBF	Simulated Body Fluid
SDF	Silver Diamine Fluoride
SEM	Scanning Electron Microscopy
SoD	Sound Dentine
CAD	Caries Affected Dentine
TEGDMA	Triethylene Glycol Dimethacrylate
UDMA	Urethane Dimethacrylate
WHO	World Health Organisation
4-META	4- Methacryloyloxyethyl trimellitate anhydride

Chapter 1

1 Literature Review

1.1 Human tooth structure

The human teeth develop and take their position into the jaw following a highly organized process. (Bartlett, 2013) They consist of an outer part, which is known as enamel for the part around the crown and cementum for the part surrounding the root surface. The cementum is involved in the attachment of the tooth with the surrounding bone (Goldberg, 2011). Below the enamel and cementum the tooth consists of dentine. Dentine is softer and more flexible than the enamel and therefore it has a supportive role. The applied forces from the enamel are transferred to dentine through the dentino-enamel junction (DEJ). The pulp lies in the centre of the tooth and contains the nerve and the blood supply of the tooth and is usually not mineralised (Bartlett, 2013; Goldberg, 2011).

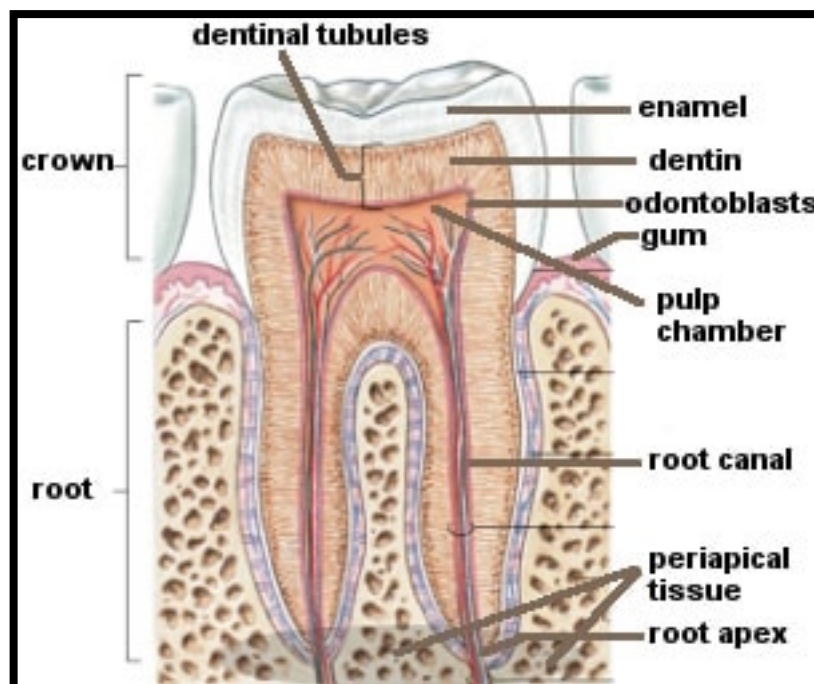


Figure 1.1 Tooth structure (Image taken from Medline Plus- a service of the U.S national library of Medicine National Institute of Health (MedlinePlus.gov))

1.2 Histology of enamel

The enamel is a highly mineralized tissue and is considered to be the hardest tissue in the human body. Enamel consists of highly organised hydroxyapatite crystallites, which form the enamel prisms (Simmer et al, 2001) and is formed during the amelogenesis process, by enamel-forming cells called ameloblasts. This process has a first stage of enamel deposition followed by a second stage, which is the maturation of enamel (Nacarino-Meneses et al, 2017).

Several enamel matrix proteins are involved in the mineralization of the enamel. The vast majority of those proteins (90%) belong to the amelogenin group. The rest of them are proline-rich, non-amelogenins, tuftelin or other serum proteins (Simmer et al, 2001).

1.3 Histology of dentine

Dentine is a hydrated biological composite structure and is considered a mineralised connective tissue (Ryou et al, 2012; Haeri et al, 2014). It is composed of 50 vol% of calcium deficient apatite. Dentine is formed by odontoblasts derived from the ectoderm. Odontoblasts can be found in the pulp tissue for life and can add some dental tissue regeneration properties and repair ability (Huang et al, 2011). These cells are responsible for the development of the predentine matrix, the matrix that contains the type I collagen, which is the organic part of dentine and represents 30% vol of its composition and finally the proteases, proteoglycans and non-collagenous proteins such as dentine sialoproteins, bone-morphogenic proteins, phosphoryns and insulin-like growth factors (Bleicher et al, 2014; Stape et al, 2018).

Various enzymes are incorporated into human dentine and are responsible for the degradation of dentine collagen matrix proteins including type I collagen. These enzymes belong to the matrix metalloproteinases group (MMPs), which is part of the calcium/zinc-dependent endopeptidases, or cysteine cathepsins (Tjäderhane et al., 2013). Odontoblasts have been found to contain some MMPs, such as gelatinases MMP-2 and MMP-9. These can be also found in the predentine/ dentine complex of fully mineralized teeth and are responsible for the disintegration of the extracellular matrix (ECM) (Boushell et al., 2008). MMPs also take part in the cell signaling by generating peptides with specific biological activities (Chaussain et al., 2009).

After the deposition of primary dentine at the time of tooth development, odontoblasts continue to gradually deposit secondary dentine throughout life if their functioning ability remains undisrupted by external factors. It is believed that in the presence of any injury-like challenges the so-called reactionary dentine is produced, not only by the surviving odontoblasts, but also by other cells that can be found in the zone below. If more severe changes occur, pulp-derived stem or progenitor cells differentiate into mineralised dentine matrix-producing odontoblast-like cells and they deposit the reparative dentine (Ricucci et al, 2018). There is a study showing that under deep caries lesions an amorphous, atubular calcified tissue can be deposited after pulp capping of carious exposures and there is no evidence of morphologically identifiable odontoblast-like cells. This is coming in contrast to the generally accepted view that stem cells induce a new generation of odontoblast-like cells, which produce reparative dentine. In that study, the authors demonstrated that the stem cells might not have received adequate epigenetic signals, such as the lack of cross talk with cells derived from the ectoderm (Ricucci et al, 2014).

Another important characteristic of the dentine are the dentinal tubules. The tubules may vary in number and size, depending on the area of the tooth, with lower density near the dentine-enamel junction (DEJ) and highest number as well as larger diameter near the pulp. They are formed by the mineralization mechanism of odontoblast cells and represent tracks that start from the DEJ or cementum up to the pulp chamber (Haeri et al, 2014).

1.4 Dental Caries

1.4.1 What is dental caries - Definition

The term dental caries is being used to describe both the clinical manifestation of caries as well as the carious lesion (Young D. et al, 2015). Dental caries reflects the symptom of the disease, either it is past or ongoing (Fejerskov et al, 1997). Generally, demineralization or destruction of dental hard tissues as a result of bacteria comes under the term “dental caries” (Braun, 2017).

1.4.2 Dental caries - Prevalence

Dental caries is a multifactorial, sugar and plaque-dependent chronic disease that affects many individuals (Pitts et al, 2017). According to WHO dental caries affects the majority of adults worldwide and 60-90% of schoolchildren (Petersen et al, 2008; WHO, 2016). It is considered to be one of the most common chronic diseases affecting the populations around the globe. Impressively, according to the 2016 Global Burden of Disease Study, dental caries that remains untreated was found to be the most prevalent among 328 conditions (Anon et al, 2017). The prevalence is relatively high in America but still quite low in African countries (WHO, 2002; WHO Country/Area Profile). According to the 2013 Children’s Dental Health Survey, caries into dentine affects 40% of the 5-year old children and 46% of the 15 year olds in the UK (Pitts, Chadwick, 2013).

The presence of dental caries can have a negative impact on the social life and on the quality of life due to the pain and discomfort that it can cause (J de Paula et al, 2015).

In addition to the above, the annual expenses for the management of dental caries in the UK currently amount to 3.4 billion pounds for the National Health Service (NHS), increased by an additional 2.3 billion pounds upon including private dentistry. (Godson et al, 2018)

1.4.3 Aetiology of dental caries

Dental caries is undoubtedly a disease that can be prevented. (Godson et al, 2018) One of the main factors responsible for the development of dental caries is the biofilm created in the tooth surface. The biofilm, known as dental plaque, if left on the teeth, can lead to the disturbance of the balance between pathological and protective factors in the oral cavity and subsequently to demineralization of the enamel and creation of the caries cavity (Pitts et al, 2017).

Factors associated with dental caries aetiology include the increased consumption of sugars and inadequate exposure to fluoride (WHO 2010, 2015). Salivary secretion rate can also play an important role (Fejerskov et al, 1997). Recent studies are also investigating the impact of genes in caries susceptibility. Matrix metalloproteinases (MMPs) and their inhibitors have been implicated in the dental caries process and the levels of heritability have been shown to reach 40% to 60% (Lewis et al, 2017).

Dental caries is a dynamic process. The bacteria residing the biofilm cause differences in the pH of the oral cavity. Specifically, the acid produced by the bacteria causes a drop in the pH of the oral cavity, leading to the dissolution of hydroxyapatite of enamel and dentine surfaces (Van Strijp et al, 2003).

There are three hypotheses associated with the aetiology of dental caries:

1. Specific
2. Non-specific and
3. Ecological (Takahashi & Nyvad, 2011)

In addition to the above, there are other factors involved in dental caries aetiology, having to do with the social and behavioural status of the people. People from lower income and education groups are considered to be at a higher risk of developing caries, due to the higher level of sugars in their diet and limited access to dental care (Petersen & Kwan, 2010).

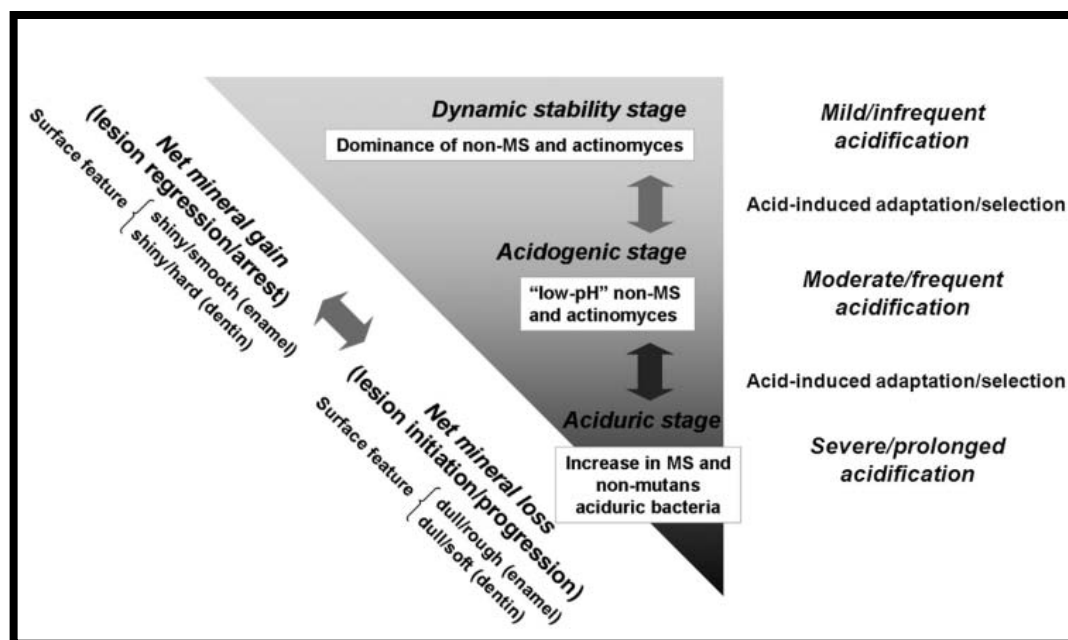


Figure 1.2 The caries process according to an extended caries ecological hypothesis (modified from Takahashi and Nyvad, 2008)

1.4.4 Caries into enamel

The occlusal surface of the teeth is highly affected by dental caries due to the presence of pits and fissures acting as plaque accumulating factors. As a result, they increase the difficulty to disturb the biofilm formation and remove the plaque. Occlusal discoloration can be seen on the pits and fissures of teeth as an early sign of dental caries. This can be due to staining and is known as non- cariogenic discoloration (non-CD). Non- CD refers to the attachment of staining chromogens to sound tooth surfaces. Cariogenic discoloration (CD) on the other hand, represents the discoloration of porous structures due to bacterial metabolites and mineral loss from the enamel surface (Lee et al, 2018).

When the caries process is initiated in the enamel, mineral loss takes place, starting from the surface of the tooth. Specifically, if dental plaque remains in the tooth surface without being removed for two weeks, the “white-spot lesion” can be seen in the enamel surface. If this lasts for a longer period of time (such as 3-4 weeks), then the enamel surface presents a more opaque and matte lesion, visible even without air-drying of the surface (Kidd, Fejerskov, 2004). White or brown opacities can be observed on the occlusal surfaces as a result of the difference in the refractive index between the sound and carious areas of the tooth (Lee et al., 2018).

1.4.5 Caries into dentine

Histologically and clinically, the caries lesion of the dentine has been characterised as having two layers. The first layer is called “the infected zone”. This is the outer zone of the lesion (which stains with caries-detector dyes) where the dentine is found highly demineralised and the collagen is denatured and heavily infected with bacteria.

The second one, which lies beneath the infected zone, is characterised as the “affected zone”. In the affected zone, which does not stain with a caries-detector dye, the dentine is demineralised, however the collagen is still intact and minimally infected (Braun et al, 2017).

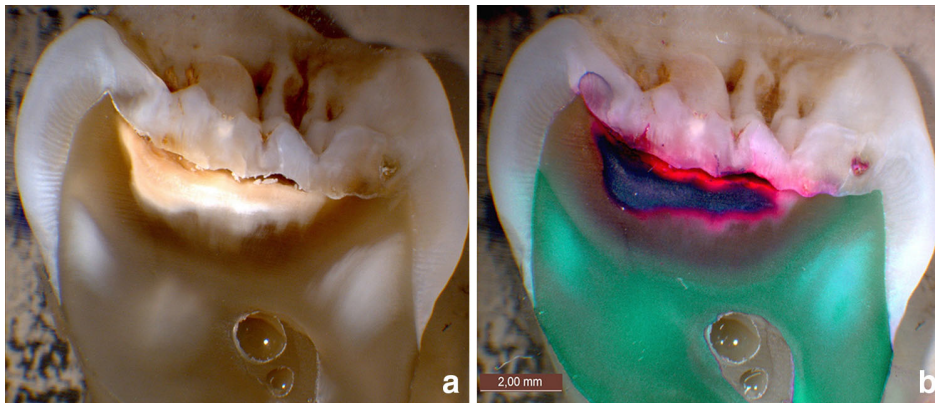


Figure 1.3 Ground section of a molar before (a) and after (b) staining with rhodamine B, fuchsin and acetic light green dye to improve differentiation between sound and carious dental hard tissues (Braun et al., 2017)

1.4.6 Recurrent caries

The term “recurrent caries” is used in order to describe the caries lesion developed in the margins of a restoration. This lesion occurs after the initial caries has been removed and the cavity has been filled with a restorative material (Mjor et al, 2005). It is therefore referred to as “secondary caries lesion”.

Some approaches have been proposed over the years for prevention from secondary caries lesions. Placing the margins of the restoration in an area that would be easily accessed by the toothbrush in order to maintain appropriate oral hygiene has been one of these approaches for many years. More recently though, the approaches lean

towards fluoride releasing restorative materials, which have been proposed for the prevention of secondary caries in the margins of restorations (Leung et al, 2005).

1.5 Management of dental caries

For the management of a caries cavity, several different methods have been proposed. Complete removal of the carious demineralized dentine followed by the restoration of the cavity with filling materials has been the traditional way of managing dental caries for a long period of time (Bjorndal et al, 2010).

However, several studies have shown that these conventional methods can lead to unnecessary tooth structure loss and greater risk of pulp involvement or symptoms of pulpitis following restoration (Ricketts et al, 2013), while non-vital teeth have been shown to be more prone to fractures (Cohen et al, 2006). Further limitations of the more invasive approach include the need to use local anaesthesia, handpieces for the caries removal and highly trained dental professionals (Dorri et al, 2017).

Stepwise excavation, partial caries removal, the Atraumatic Restorative Technique (ART) and the Hall Crown Technique are increasingly more evidence based. According to these approaches only part of the caries lesion is being removed, while the harder more intact caries is left behind in order to avoid pulp exposure (Ricketts et al, 2013; Innes et al, 2011; Holmgren, 2013). The success depends on the caries that is left behind being sealed in.

1.5.1 Stepwise excavation

In stepwise excavation the soft carious dentine is removed from the margins and as much as possible also removed from the cavity, with caution in order to avoid exposing the pulp. The remaining lesion is covered with an antimicrobial material (usually calcium hydroxide) and a few months later the cavity is accessed again and the remaining dentine is reviewed (Bjorndal et al, 2008). Hayashi et al in their systematic review found that stepwise excavation leads to a high clinical success rate, the remaining lesion was found hard and remineralised in most of the cases and pulp vitality was preserved (Hayashi et al, 2010).

1.5.2 Atraumatic Restorative Technique (ART)

The Atraumatic Restorative Technique (ART) was implemented in order to overcome the above-mentioned disadvantages of the conventional more invasive approaches of caries management. The concept behind this technique is that the carious dentine is removed with the use of excavators (hand-instruments) only, followed by restoration with adhesive materials, such as composite, GIC or RM-GIC (Tyas, 2000). This results in a more conservative way of managing the carious lesion, preserving the maximum amount of sound tooth structure.

Adequate sealing of the remaining, intact, remineralisable dentine is important in order to prevent mineral loss out of the lesion and acid penetration from the bacteria of the biofilms (Dorri, 2017 Cochrane Review). Studies have shown higher levels of success in partial caries removal compared to the stepwise excavation (Maltz, 2012).

1.5.3 The Hall Technique

The Hall Technique has been another minimally invasive approach of restoring the primary teeth, which was first implanted by Dr Hall, who was restoring carious primary molars without any caries removal, local anaesthesia or tooth preparation for years having successful outcomes. This technique had initially caused controversy due to the lack of understanding how leaving all the carious tissues and the associated bacteria below a crown could lead to favorable outcomes. Nowadays, there is evidence that can explain the above results along with the results of other minimally invasive techniques based on the fact that caries concept has moved from that of an infectious disease, towards that of a biofilm- driven disease (Innes et al, 2017).

According to Hall Technique, when a carious primary tooth has no clinical or radiographic symptoms, the tooth can be sealed with a preformed metal crown without the prior removal of caries from the cavity. Space can be created with the use of an orthodontic separator and the crown is placed without prior use of a handpiece or local anaesthesia.

It has been shown that sealing the caries below the preformed metal crown can slow down the progress of the lesion (Innes et al, 2011).

1.6 Materials used so far for restoration of cavities

Several materials have been introduced as fillings for the restoration of the cavities.

The most widely used materials are:

1. Amalgam
2. Glass ionomers
3. Compomers
4. Resin composites

1.6.1 Amalgam

Amalgam has been used as a restorative material since the early days of restorative dentistry due to its long-term longevity, easy placement technique and handling and also because of its low cost (Bharti et al, 2010). The last years though, the use of amalgam is being questioned not only because of the presence of mercury in the composition, but also due to the lack of aesthetics of the amalgam restorations.

According to the American Dental Association (ADA), dental amalgam is defined as an alloy containing 40-45% of mercury, 40- 70% silver, 12- 30% tin, and 12- 24% copper along with other metallic elements added to improve physical and mechanical properties, such as 0-4% indium, 0.5% palladium and up to 1% zinc (ADA Statement on Dental Amalgam, 2011).

Studies have shown that the dental amalgam seems to have better longevity and lower recurrent caries rates compared to the composite materials (Moraschini et al, 2015; Opdam et al, 2005).

Research has found no direct impact on patients' health. Ucar et al. in their systematic review found that the mercury released from dental amalgam restorations is not associated with any toxicological effects or systemic disease, while some allergic reactions to amalgam have been reported, but the likelihood is extremely low (Ucar et al., 2011). Dental amalgam though, comes in the second place among the sources of mercury use in the European Union and despite the well improved dental amalgam capture and disposal technologies, these don't seem to be implemented in every practice (Fisher et al, 2018). As a result, attempts have been made over the last years to reduce the use of amalgam, as it can affect and pollute the environment.

Specifically, in 2013, the United Nations Environment Programme (UNEP) signed the Minamata treaty agreement in order to minimize and gradually completely withdraw the use of amalgam in dentistry (UNEP, 2013). The Minamata Convention came into effect in August 2017 and is an international legally binding agreement for the protection of the environment and public health from any spread of mercury or its components (Fisher et al, 2018).

Some of the main issues addressed by Minamata Convention include not only the phase-out of mercury mines and banning of any new ones, but also addresses the current use and gradual termination of mercury use in a number of products and processes, including dental amalgam. The Convention also deals with the interim storage of mercury and its disposal when it becomes waste, sites contaminated by mercury as well as health issues (<http://www.mercuryconvention.org>)

According to the last regulations, from the 1st of July 2018, dental amalgam "shall not be used for dental treatment of deciduous teeth, of children under 15 years and of pregnant or breastfeeding women, except when deemed strictly necessary by the dental practitioner based on the specific medical needs of the patient" (Article 10(2) EU Regulation (EU) 2017/852 on Mercury).

1.6.2 Glass Ionomers

The glass ionomers are another category of restorative materials along with the amalgam and the composites. They were first implemented in the early 1970's and had some advantages compared to the amalgam, which was the main restorative material at this time. These advantages included better aesthetics and adhesion to the tooth structure. Glass ionomers display properties such as a similar thermal expansion compared to that of a tooth, ability for chemical bonding to both enamel and dentine, while they are biocompatible at the same time (Dhar et al, 2015).

Glass ionomers come as luting cements, base/ lining materials as well as restorative materials. The composition of glass ionomers is based upon the presence of polycarboxylate and silicate materials, which are able to create ion binding with the hydroxyapatite of the enamel and dentin (Saito et al, 1999). The polymerization of the GIC's comes following a neutralisation reaction between fluoro- alumino silicate glasses and a polyacrylic acid solution (Young et al, 2002).

Glass ionomers are easy to handle, do not require optimal moisture control or multiple application steps. Apart from the better aesthetic properties compared to the amalgam, they also release fluoride, which is considered to be their main advantage. Fluoride release has been shown to continue for at least one year following placement of the material (Dhar et al, 2015) and is a property that makes GIC restorations less susceptible to recurrent caries. GIC's also display thermal compatibility to tooth enamel and low toxicity (Lohbauer et al, 2010).

Although glass ionomers create a chemical bond to enamel and dentine, their strength is lower compared to the other two traditionally used restorative materials, amalgam and composite. Another disadvantage of the GIC's is the wear of the restoration, as their contact free area (CFA) wear is five times higher than amalgam and three times higher than composite (Kunzelmann et al, 1996). Furthermore, according to the literature their use for the restorations of Class II cavities in primary molars is not indicated (Chadwick et al, 2007).

1.6.3 Resin-modified glass ionomers

One category of the glass ionomer restorative materials is the resin-modified glass ionomers (RMGIC's). The resin-modified glass ionomer cements were implemented in 1980's in an attempt to create a material that could add some of the advantages of the composites, such as aesthetic and mechanical properties, into an improved glass ionomer material (Meyer et al, 1998).

The modification of the glass ionomers was achieved by including a small amount of polymerisable resin composite inside the conventional glass ionomer structure. The replacement of the polyacid with a modified polyacid combined with unsaturated groups and the incorporation of polymerizable hydrophilic resins resulted in the creation of the resin-modified GIC's or otherwise called light-cured GIC's (Meyer et al, 1998).

The RMGICs initially set through resin polymerization, a quality that differentiates them from the conventional GICs, which set via an acid-base reaction. This way, the setting time is improved without an impact on the handling properties of the material (Chadwick et al, 2007). Setting of RMGIC's takes place through a chemical and/ or a light activated process as well as an acid/ base reaction (McCabe et al, 1998).

The RMGIC's usually consist of a fluoro-alumino-silicate glass mixed with a liquid such as HEMA (Hydroxyethyl methacrylate), a polyacid and water (Young et al, 2003).

Due to the release of chemical components such as fluoride and metallic ions, these materials have been shown to present with antimicrobial properties. These properties can be a result of the low initial pH as well (Farrugia et al, 2015).

Overall, resin modified glass ionomer cements have significantly improved the physical properties of these materials and they have the advantage of quick setting thanks to the use of visible light exposure. Resin modified glass ionomers have been manufactured and packaged in order to promote easier handling and placement of the material, overall user convenience, and improved properties compared to the conventional glass ionomers (Croll et al, 2010).

1.6.4 Compomers

Polyacid modified composite resins or otherwise referred to as compomers, are another category of restorative materials that came in the market in mid- 1990's (Meyer et al, 1998).

Compomers aimed to combine some properties of the composites along with some properties of the GIC's, such as fluoride release, good aesthetics and simplicity in the handling process of the material. The fluoride release can be triggered by the presence of moisture. Many compomers consist of 72% strontium fluorosilicate glass and a mean size of 2.5 micrometers of particles (Nicholson et al, 2007).

Compomers' properties are also limited due to polymerisation shrinkage, which can be compensated by water sorption (Huang et al, 2002).

According to the literature, no statistically significant difference was found in the longevity of compomer Class I restorations when compared to this of the amalgam restorations, however they were found to require more often replacement as a result of secondary caries adjacent to the restoration (Dhar et al, 2015).

1.6.5 Dental composites

The recent shift on the dental restorative approaches towards more conservative techniques and minimal intervention dentistry has led into phasing down the mechanically- retained filling materials, such as amalgam in favour of adhesive materials (Fisher et al, 2018). Consequently, the dental composites, as adhesive restorative materials, have managed to reduce the need for mechanical retention, giving the opportunity for more conservative as well as aesthetic restorations.

The history of dental composites starts back in 1955, when Buonocore suggested that etching (conditioning) the enamel surface with phosphoric acid could improve the resin adhesion into enamel (Buonocore et al, 1955). Since then, a lot of research has been done in order to improve the properties of the dental composite materials. Dental composites can be differentiated by changes in their composition according to their requirements as restorative materials, fissure sealants, cements or provisional materials (Ferracane et al, 2011). One of the recent innovations has been the reduced in size reinforcing filler, which adds to the composites greater wear resistance, important for the posterior restorations and it makes them easier to polish, improving the aesthetics especially for the anterior restorations (Ferracane et al, 2011).

There is evidence according to the current literature that the composite restorations are successful for Class I cavities in primary teeth but only one randomized controlled trial showing evidence of success of Class II composite restorations in primary teeth that are about to exfoliate within a two year period (Dhar et al, 2015).

1.6.5.1 Composition of dental composites

The dental composites usually consist of glass particles dispersed into hydrophobic methacrylate monomers. These materials polymerise with light exposure (Young et al, 2003).

The composition of the commercial dental composite materials commonly includes four different components:

1. An organic polymer matrix, produced by mixing for example UDMA (Urethane Dimethacrylate), BisGMA (Bisphenol A glycol dimethacrylate) and TEGDMA (Triethylene glycol dimethacrylate), which are dimethacrylate monomers.
2. Inorganic reinforcing fillers usually radiopaque glass or ceramic (such as silica, lithium aluminum silicates, hydroxyapatite and boron silicates) (Kundie et al, 2018)
3. Silane coupling agents that can increase the bonding between the fillers and the organic matrix (Lung et al, 2012)
4. The free-radical initiator-accelerator system (Wu X., 2010; Fong H., 2004; Hosseinalipour M., 2010; Klapdhor S., 2005)

The physical properties of the resin composite materials are mainly characterised by the filler system (Erickson, 1994).

1.6.5.2 Dimethacrylate monomers

Dimethacrylate-based composites have been the materials of use for various restorations in dentistry. Their application includes dental adhesives, pit-and-fissure sealants, and can be combined with silane-coated glass fillers to render the most widely used esthetic direct restorative material, as well as cementation agents and veneering materials.

The monomers found in the composition of dental composite materials are usually crosslinking dimethacrylates. These can be bisphenol A glycol dimethacrylate (Bis-GMA), TEGDMA (triethylene glycol dimethacrylate), UDMA (Urethane Dimethacrylate), which is a product of 2,2,4 (2,4,4)-trimethylhexyl diisocyanate, and 2-hydroxyethyl methacrylate, and Bisphenol A Ethoxylated Dimethacrylate (Bis-EMA). Most of the times mixtures of these monomers are used (Sideridou et al, 2005).

Bis-GMA is the monomer for most of the commercial composites and it is more often seen as a combination with a low viscosity monomer, such as the diluent TEGDMA, which can be used for reduction of the viscosity of the liquid phase, in order to increase the material conversion and to enable easier handling (Gajewski et al, 2012).

The addition of TEGDMA though can lead to an increase in water sorption (Sideridou et al, 2007) and reduce of the mechanical properties of the composite (Goncalves et al, 2010).

In order to compensate the limitations of BisGMA-based systems different high molecular weight monomers have been developed and added in materials recently introduced in the market. UDMA (Urethane dimethacrylate) materials became increasingly common, due to the monomer's low viscosity and high flexibility in relation to BisGMA (De Paula et al, 2015). These composites present with higher flexural strength, elastic modulus and hardness, which are some of the properties found in UDMA polymers.

Another monomer sometimes used instead of BisGMA is its ethoxylated version, known as BisEMA (ethoxylatedbisphenol A dimethacrylate). BisEMA has a higher molecular weight (MW=540 g/mol), but lacks the strong secondary molecular interactions given by hydroxyl groups. The lack of molecular interactions reduces its viscosity and results in higher degree of conversion giving this way better mechanical

properties (Sideridou et al, 2005).

There are also various monomers that have been introduced in the market, having higher molecular weight with an aim to reduce composite shrinkage. The modified urethane dimethacrylate resin DX511 has been shown to reduce shrinkage due to its increased molecular weight compared to bis-GMA and UDMA. The urethane monomer TCD-DI-HEA can produce lower polymerization contraction stress than other low shrinking composite materials (Marchesi et al, 2010). The dimer acid monomers also present with a quite high molecular weight, and have been shown to have high conversion of carbon double bonds while undergoing lower polymerization shrinkage than bis-GMA-based systems (Ferracane et al, 2011).

PPGDMA or Poly-(propylene glycol) dimethacrylate is an alternative high molecular weight diluent monomer, which has recently been used in experimental composites. Due to its high molecular weight and flexibility, its use as a monomer can promote a greater monomer conversion, while it has significantly lower double bond concentration compared to other monomers (Walters et al, 2015; Ben Nuba et al, 2014).

1.6.5.3 Inorganic fillers

The filler is added to a composite to promote polymer properties. It usually consists of different compositions, sizes and size distributions of glass or ceramic particles, whiskers, fibres, nanotubes and nanoclusters (Kundie et al, 2018).

The size of the inorganic fillers is particularly important for the composites as it affects many composite properties. Smaller size of filler particle can lead to better aesthetics, while the composite can be polished easier. On the other hand, increase in the size of the filler particles can add strength to the material. Other properties affected by the filler size are polymerisation shrinkage and depth of polymerisation (Dhar et al, 2015).

More recent advances are the nanofillers that have been introduced in new composite materials, with a range between 0.5% and 40% by weight in the last adhesive systems. Fillers can control the handling properties and add on the strength. They can also increase the thickness of the adhesive layer (Sofan et al., 2017).

The fillers are added to improve both the physical and mechanical properties of the resin. They reduce curing shrinkage during and after polymerization, and they improve the esthetic appearance and radio-opacity (Ferracane et al, 2011).

1.6.5.4 Silane coupling agents

Silane coupling agents are used in dental composites to promote adhesion. Silanes are effective in enhancing adhesion between resin composite and silica-based ceramics. They don't lack disadvantages though and their main clinical problem as adhesion promoting agents is the gradual deterioration of bonding in the oral environment (Lung et al, 2012). The silane usually found in dental restorative resin materials is 3-methacryloxypropyltrimethoxysilane (γ -MPS) (Kundie et al, 2018).

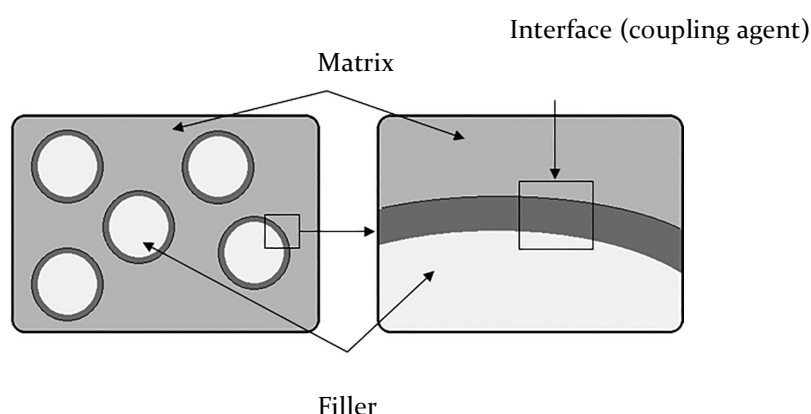


Figure 1.4 Schematic drawing of filler–matrix interphase (Kundie et al, 2018)

1.6.5.5 Initiator-accelerator system

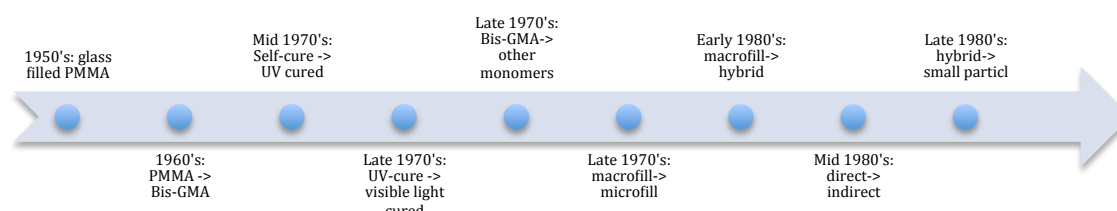
The most common initiator is CQ (Camphoroquinone) (Stansbury et al, 2000). The CQ initiates the polymerization process in the presence of a light source. CQ is commonly used photo-initiator with tertiary amine as a co-initiator in order to enhance the polymerization process (Maciel et al., 2018). When CQ absorbs light in wavelength between 430 and 510nm (blue region of visible light spectra) with a maximum absorption wavelength at 468 nm, then a photo-excitation complex with a tertiary amine is created and two free radicals are generated. These radicals are

amino and cetyl. This is considered a hydrogen abstraction photo-initiation (Asmusen et al, 2009).

The polymerization process starts from the amino radical. The light absorption spectra of CQ represents one of the main characteristics that give an advantage to this material, as it matches the spectra of the blue LEDs, which are among the most used curing lights. As a result, the CQ/amine system can be considered one of the most effective photo-initiator systems for a composite material (Sim, 2012).

Evolution of Dental Composites

1950's-1980's



1990's-2010

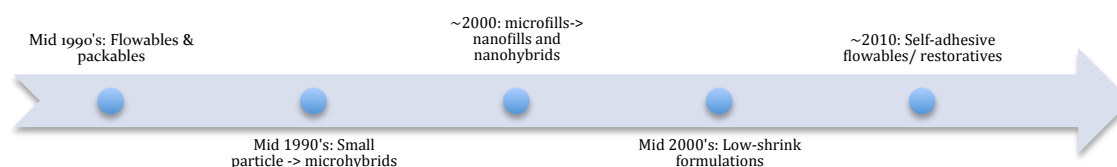


Figure 1.5 Evolution of dental composites 1950's- 2010 (Ferracane, 2011)

1.6.6 Dental Adhesives

The dental adhesives can be classified into the following main groups:

1. The three- step etch & rinse adhesive systems (3E&Ra's), which include etch, primer and bonding agent in three different bottles
2. The two- step etch & rinse adhesive systems (2E&Ra's), where the primer and bond are in the same bottle
3. The two- step self-etch adhesives, where the etch and primer are in the same bottle
4. The one- step self- etch adhesives, which in one bottle combine self-etching primer and bonding agent (Peumans et al, 2014; Sofan et al., 2017).

This classification is based on the need for prior application of etch (usually phosphoric acid) and primer or not. In the etch & rinse approach phosphoric acid is used for the conditioning of enamel and dentine, which can create deep etch-pits in enamel and can demineralise the dentine.

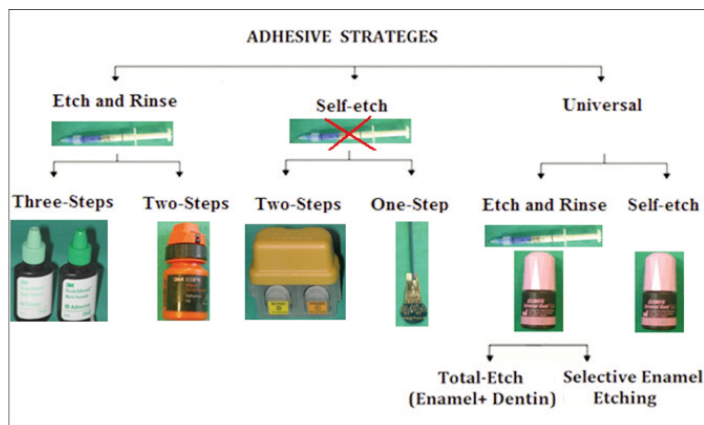


Figure 1.6 Adhesive strategies. (Sofan et al., 2017)

Figure 1.6 presents the different adhesive strategies as described above, depending on the need for etch, primer or bond, starting from the etch & rinse approach with the three steps, followed by self-etch, universal and single-step self-etching adhesives.

1.7 Problems with current materials

There are several reasons that lead to the failure of the restorations of the teeth, whether they have been restored with amalgam, composite or glass ionomer materials. One of the main reasons that these restorations fail is the micro-leakage phenomena in the margins of the restorations that can cause recurrent caries. It is the material that is being used though, that can determine the bacterial activity and growth in the interface with the tooth (Cazzaniga et al, 2015; Ionescu et al, 2015).

The higher number of recurrent caries is still seen in the composite restorations of the posterior teeth and these restorations have less longevity compared to the amalgam (Moraschini et al, 2015; Bernardo et al, 2007). Secondary caries is seen more often in the adhesive restorations, due to polymerization shrinkage, which leads to bacteria penetration as a result of micro-gap formation in the interface of tooth and composite material (Nedeljkovic et al, 2015).

Another reason for failure of the current composite restorations is polymerisation shrinkage, as it results in alterations in the interface between material and tooth. The outcome of polymerisation shrinkage is a gap between material and tooth that can enable the microleakage phenomena, can lead to fracture of the material or fracture of the tooth. These unfavourable results may cause discomfort and post- operative sensitivity for the patients (Shah et al., 2014).

In addition to the above, the longevity of the adhesive restorations is affected by the fact that they still remain technique sensitive and more complicated to place (Tobi et al, 1999) requiring optimal oral cavity moisture control and clinicians that are well trained in placement of composite restorations. Especially when it comes to paediatric patients, achieving an adequately dry cavity, full caries removal and multiple steps in placement of a restoration can be even more challenging and at times not possible at all.

The main reason for replacement of the amalgam restorations, is the fracture of the restoration, the difference though, is not statistically significant compared to the composite (Moraschini et al, 2015; Bernardo et al, 2007).

The glass ionomers have the lowest frequency of recurrent caries in the margins of the restoration, which can be a result of their antibacterial properties, however, their

mechanical properties remain poor and they present with low fracture strength and wear (Xie et al, 2000). As a result, the longevity of the GIC restorations is lower compared to the other two materials.

Amalgam	Glass Ionomer	Composite
+ Low cost	+ Low cost	- Higher cost
+ High strength	- Low strength	+ High strength
+ Lowest failure rates	- High failure rate	- Medium failure rate
+ Less recurrent caries	+ Less recurrent caries	- Recurrent caries
- Not aesthetic material	- Acceptable aesthetics	+ Excellent aesthetics
- Contains mercury	+ F ⁻ release	- Not antibacterial
- Now banned in children		- Technique sensitive
		- Microleakage

Table 1.1 Advantages & disadvantages of current restorative materials

1.8 Bonding to tooth structure

1.8.1 Bonding mechanisms over the years

The first innovations in bonding of the dental composites came more than 60 years ago by Buonocore who suggested that resin could bond better into acid etched dentin.

Since then, the bonding systems have had several approaches until they reached the way that they work today (Buonocore et al, 1955; Kugel et al, 2000).

New, less conventional restorative approaches and adhesive materials have become available in the market, allowing not only highly aesthetic but also conservative restorations. As a result, the success of most restorative procedures is mainly relying on adhesive systems providing optimal and durable bonding to tooth substrates (Malaquias et al, 2018).

Three approaches have been described for the bonding into the tooth tissue. One approach is the “etch & rinse” approach, second is the “self-etch” and the third is the “resin-modified” (glass-ionomer) approach (Van Meerbeek et al, 2003).

The main mechanism of the adhesion process of resin into the tooth surface is based on the exchange of inorganic calcium phosphate that may precipitated within collagen tubules for the synthetic composite material (Van Meerbeek et al, 2011). With the use of the etching agent, the acid is demineralizing the dentine and enamel substrates leading in an increased porosity of the surface. This porous surface is subsequently filled with a primer, which is covered by a hydrophobic agent (bond) to ensure bonding with the resin composite material (Van Meerbeek et al, 2011).

1.8.2 Bonding to enamel

Etching the enamel usually with 35-40% phosphoric acid has been a successful enamel bonding mechanism over the years (Erickson et al, 1994).

The “etch & rinse” mechanism in the enamel surface starts with the selective dissolution of the hydroxyapatite crystals of the enamel through the etching (conditioning), something that creates microporosity and etch-pits. This microporosity can be penetrated by the monomer of the composite-resin system (Erickson et al, 1994).

After the bonding agent has been applied, two categories of “resin tags” are created inside the above-mentioned etch-pits: macro-tags and micro-tags. The first ones cover the area around the enamel prisms while the latter come as a result of resin infiltration within the etch-pits of the etched enamel prisms (Van Meerbeek et al, 2003).

1.8.3 Bonding to dentine

The bonding into dentine surface has been more challenging over the years, since dentine's composition differs compared to the enamel. Dentine consists of only 45% of inorganic part compared to the 95% of the enamel, and also dentine's organic part mainly consists of collagen in contrary with the regularly arranged hydroxyapatite crystals of the enamel (Aasen et al, 1990).

Dentine structure also includes the dentinal tubules that contain fluid –something that can negatively affect the bonding- and differ in number and size the more we reach the pulp. Adequate bonding into dentine structure, therefore, has been more difficult to achieve (Swift et al, 1995).

The main principal, upon which dentine bonding is based, is the formation of a hybrid layer between the dentine surface and the composite material. (Nakabayashi et al, 1982) The hybrid layer is created when the monomers of the composite are being polymerized inside the dentine collagen network, forming a micromechanical bond (Erickson et al, 1994).

In the dentine substrate, removal of the minerals following the application of acid etch exposes the collagen mesh and noncollagenous proteins. Commercially available adhesive systems fail in dentine infiltration (Carvalho et al, 2005) as a result many sites of dentine may remain exposed. The matrix metalloproteinases (MMPs) and cysteine cathepsins, which are involved in the degradation of exposed collagen, remain during the bonding procedure via an endogenous proteolytic mechanism, even in the absence of bacteria.

Generations of Bonding Systems

1st Generation	1956: Buonocore shows that use of a glycerophosphoric acid dimethacrylate-containing resin could bond to etched dentine
2nd Generation	Late 1970's : halophosphorous esters of unfilled resins such as bisphenol-A glycidyl methacrylate, bis-GMA, hydroxyethyl methacrylate or HEMA are added to the bonding systems
3rd Generation	The smear layer is partially removed by the acid etching of the dentine. The primer contains hydrophilic resin monomers, including hydroxyethyl trimellitate anhydride or 4-META, and biphenyl dimethacrylate or BPDM
4th Generation	1980's : Complete removal of the smear layer, use of the total-etch technique
5th Generation	Two different types of adhesive materials: 1. "one-bottle systems" and 2. the self-etching <u>primer</u> bonding systems
6th Generation	Bond to enamel and dentin using only one solution. These materials should be a one-step bonding system
7th Generation	Late 1999- Early 2005 : One bottle, self- etching systems
8th Generation	2010 : Introduction of the non-sized fillers, with an average particle size of 12 nm, shown to have a positive impact on bonding due to the penetration of resin monomers and the hybrid layer thickness

Table 1.2 Generations of Bonding Systems (Kugel et al., 2000; Sofan et al, 2017)

1.8.4 Effect of caries in bonding ability of the materials

Another challenge in the bonding ability of the materials can be the presence of caries in the cavity. The dentine caries lesion is characterized by the presence of two zones. The outer zone constitutes the "infected dentine" and the inner zone is the "affected dentine" (Pinna et al, 2015).

The carious dentine is partially demineralized, while the dentinal tubules contain mineral crystals. This could benefit the deeper etching of intertubular dentin but can have a negative impact on the resin tag formation during bonding (Yoshiyama et al, 2002).

1.8.5 Shear bond tests in human dentine

Several methods have been used in order to assess the bonding between the tooth and material interface. The ISO guidelines for testing the adhesion to tooth structure, suggest three tests: shear bond tests, microleakage and micro-tensile tests (ISO guidelines for: Testing of the adhesion to tooth structure).

Shear bond tests in human dentine have been commonly used in order to assess the different materials and bonding systems. They work by placing a composite system into a flat dentine surface of a tooth, followed by the application of force until the bond between the tooth and the composite breaks.

Their validity though has been questioned, due to the many factors affecting the results as well as the fact that they don't take into consideration several aspects of clinical performance (Olsson et al, 1993; Swift et al, 1995).

Among these aspects are the mechanical properties of the three-dimensional cavity, the dentinal fluid and the pulpal pressure and also the differences in bond strength when we go close to the pulp, which is reduced due to the difference in density of the dentinal tubules and the increase in the dentinal fluid. Micro-leakage is also not being taken into account when doing these tests (Kugel et al, 2000).

1.9 Ideal properties of a new composite material

As described above, current materials are not providing the optimum set of properties to allow adequate restoration of teeth. This is particularly in children, when adequate isolation is often not possible to achieve. During recent years, attempts have been made to develop new materials based on composites that provide all the desired properties for better performance and less time consuming application.

An ideal material should have good strength to achieve longevity of the restorations, biocompatibility, good aesthetic properties and colour stability. It should also have a remineralising potential to help repair affected dentine remaining in the cavity below the restoration and also be resistant to recurrent caries adjacent to the restoration. Along with the above qualities, a modern material should be easy to place, without

time consuming and technique sensitive application, without the need for prior use of etch and bonding and without the necessary optimal moisture control that the current composites demand.

Some of the above features have been developed in recent novel experimental composite materials.

1.10 New novel composites- Recent advances in dental composites

If the composite materials did not require the etch and rinse step as well as the addition of a bonding agent, that could make the clinical application and use easier and more time effective for both the clinician and the patient. The last years, there have been a few studies conducted towards that direction, involving self-adhesive as well as remineralising composite materials.

1.10.1 Self-adhesive dental composites

Most of the commercial composites that are currently used, are hydrophobic materials and do not possess self-adhesive properties. Therefore, they require the application of additional bonding agents. (Erickson et al, 1994) Studies have been conducted over the years in an attempt to introduce composites with self-etching and self-adhesive properties. Self-adhesive composites are aiming to bond without the use of etch or bond prior to the placement of the material (Poitevin et al, 2013). These composites could benefit the clinical performance, as they require less time for their application, by avoiding the steps of etch & rinse and placement of the bonding agent.

The self- adhesives contain more acidic monomers. These systems can have one or two steps of application depending on whether the bonding agent includes the self-etching / primer solution or it is separated from it. A commercial self-adhesive composite reported in the literature (Vertise Flow, Kerr, Orange, CA, USA) contains phosphoric acid ester methacrylate and glycerol- phosphate dimethacrylate (GPDM) as functional monomers. It has been found that self- adhesive composites contain acidic monomers, such as 4-META or glycerolphosphate dimethacrylate (GPDM), which through ionic interactions can promote the adhesion to positively charged

calcium ions, or possibly also positively charged amino- acid groups in collagen (Ferracane et al, 2010).

The bond achieved with these one-step adhesives has been shown to be more susceptible to hydrolytic degradation over time due to the hydrophilic nature of their monomers (Cardoso et al, 2011). In addition, none of the different adhesive systems available at the moment is capable of preventing nanoleakage and this can explain the presence of secondary caries, loss of retention around composite restorations and discolouration of the margins (Van Meerbeek et al, 2007).

Not many resources can be found in the literature though, regarding the adequacy of the bonding between this type of composites and the tooth structure and only two commercial composites so far are self-adherent (Fusio Dentin Liquid- Pentron, Vertise Flow- Kerr) (Poitevin et al, 2013; Pinna et al, 2015). Specifically for Vertise Flow, shear bond-strength tests have shown that its bonding effectiveness was lower compared to multi- step adhesives (Mine et al., 2017).

1.10.2 Antibacterial & remineralising composites

1.10.2.1 Chlorhexidine

In an attempt to reduce the failure of the current composite restorations due to recurrent caries, new composite formulations have been proposed, claiming to have remineralising and antibacterial properties. One of the materials that have been added in these new composites in order to achieve the antibacterial effect is chlorhexidine (CHX). Chlorhexidine not only works as an antibacterial agent, but also inhibits the MMP action, which then leads to the degradation of the dentine collagen after the dissolution of its hydroxyapatite (Malaquias et al, 2018). Even low CHX release has been shown to have the ability to reduce the amount of acidogenic bacteria, such as *Streptococcus mutans*, but also to reduce the biofilm viability, biofilm formation and metabolic activity and the lactic acid production (Xia et al, 2014).

Chlorhexidine however, has shown potential toxicity even in low concentrations when in contact with odontoblast-like cells (Lessa et al, 2010).

1.10.2.2 Polylysine

Another component that has recently been proposed in experimental composites with an aim to add antibacterial properties in the material is polylysine (PLS). Polylysine has been approved by the FDA (Food and Drug Administration) as a food preservative but has also been used as drug delivery carrier, endotoxin remover, biosensor, disinfectant etc and is effective against a wide spectrum of bacteria (Shih et al, 2006).

1.10.2.3 Calcium Phosphates

Calcium phosphates are of great interest for biomedical or dental applications. Calcium phosphates contain in the structure some calcium ions (Ca^{2+}), as well as phosphorous-containing groups, such as orthophosphate (PO_4^{3-}), metaphosphate (PO_3^-) or pyrophosphate ($\text{P}_2\text{O}_7^{4-}$), doped with a hydrogen (H^+) or hydroxyl ions (OH^-). The most important differences between the compounds belonging to this group results from the calcium to phosphorus ratio, which is strictly connected with

the solubility.

The composition of CaP includes the presence of mono- calcium phosphate monohydrate (MCPM) and beta- tricalcium phosphate (β -TCP), which react in the presence of water and create higher volume dicalcium phosphate precipitates. These can lead to material expansion, compensating for polymerisation shrinkage (Gandolfi et al., 2011).

Calcium phosphates are similar to the mineral part of tooth and bone and can enhance the osteoconduction and bone growth. They are also non- cytotoxic, thus biocompatible. CaPs have lower cost compared to the majority of currently used inorganic fillers. They are used in almost all possible forms, e.g. powder, granules, bulk, porous scaffolds, self bonding cements, coating, pastes, components of composite materials.

Other materials that have been used as fillers in order to achieve a remineralising role for the new composites are: hydroxyapatite (HA), Amorphous Calcium Phosphate (ACP), Tetracalcium phosphate (TTCP) and mono- and di- calcium phosphates.

1.10.2.4 Amorphous Calcium Phosphate (ACP)

The addition of Amorphous Calcium Phosphates (ACP) to the composites has been investigated. Composites containing ACP though can come up with low strength. As a result, they cannot be used in areas where increased forces apply, such as the area of the posterior teeth (Regnault et al, 2008). ACP bond strength is about 18 MPa and the maximum biaxial flexure strength of composite containing ACP is has been reported reaching 50MPa bond strength, which is similar to that of GICs (Mehdawi and Young, 2012).

1.10.2.5 Mono- and di-calcium phosphates (MCPM, DCPA)

MCPM or monocalcium phosphate monohydrate is a reactive calcium phosphate filler with hydrophilic and soluble nature. The hydrophilic element enhances the water sorption, which in return, enables the release of calcium phosphate and phosphoric acid. MCPM also promotes brushite formation, leading to re-mineralisation of the

dentine. So MCPM is adding remineralising and self-etching properties to the composite formulations (Aljabo et al, 2015).

1.11 Importance of new composite materials in paediatric dentistry

It is very important for everyday clinical practice, and for providing quality care to patients, to constantly improve materials used in dental restorations, in order to overcome their limitations.

In paediatric dentistry, this would make a significant difference for both clinicians and patients, if the time that is necessary for a filling restoration was reduced, or if the placement technique was simplified. The conditions of the clinical setting in paediatric dentistry are not always ideal, mainly due to the patient's anxiety and lack of cooperation, making it relatively hard to achieve optimal moisture control, to manage complete caries removal or place a technique-sensitive material like the current composites.

Furthermore, the implementation of a less demanding and technique-sensitive material could be a potential benefit for the less developed and deprived areas where the access to dental facilities is limited.

It is therefore essential to introduce new materials with improved properties and less time-consuming or invasive application technique, in order to make the dental visits easier for both patients and paediatric or general dentists.

Chapter 2

2 The Project – Materials & Methods

2.1 The Project- Aims & Objectives

Given the need for constant improvement of current materials, new composite formulations have been developed by the department of materials of the Eastman Dental Institute, with a view to implement self-adhesive, remineralising and antibacterial properties, leading to a less technique-sensitive application, as well as having the ability to remineralise remaining carious dentine and prevent the development of recurrent caries in the margins of the restorations. Along with the above-mentioned qualities, these new materials aim to be non-toxic and biocompatible at the same time.

The materials should have properties that can be described by the acronym **SMART**:

S M A R T →

Self-adhesive **M**echanical strength **A**ntibacterial **R**emineralsing **T**reatment of teeth

The aim of this project was:

1. First to develop a protocol for standardised testing of the shear bond strength of the new self-adhesive, remineralising and antibacterial composite formulations when tested in human dentine and in ivory dentine
2. Second to assess the shear bond strength of these formulations and how the above-mentioned properties can influence it compared to the shear bond strength of commercial materials
3. To prove that the SMART material can be placed in large cavities, with minimal removal of affected dentine
4. And finally, to assess the resin tag formation, presence of hydroxyapatite and the monomer conversion of the final version of the formulations (SU2) in comparison to commercial materials, such as Aactiva (ACTIVA™ BioActive), Fuji II-LC (GC Fuji II™ LC) and Z250 (3M).

The components of the formulations that this project is dealing with (A6, C2 and SU2) are described in tables 2.1, 2.2 and 2.5.

- For the shear bond tests human dentine and ivory dentine were used and the initial aim was to develop a standardised protocol that can be applied in order to do repeatable tests of the shear bond strength in human dentine
- For the assessment of the resin tag formation images were taken using the light microscope, the Scanning Electron Microscopy (SEM, Phillip XL-30, Eindhoven, The Netherlands) and the Confocal Microscopy
- RAMAN microscopy was employed in order to assess the presence of hydroxyapatite in the material/ dentine interface
- Finally, for the assessment of the monomer conversion and the chemistries of the different materials the ATR FTIR was employed

2.2 Materials

2.2.1 Monomer of the new formulations (A6, C2, SU2)

The new formulations have been created to combine remineralising, antibacterial and self-adhesive properties, with an aim to reduce the steps of the filling application process and also to enhance remineralisation of any remaining carious dentine in the cavities. Furthermore, they aim to provide adequate sealing of the cavity even in the case of partial or no caries removal, or in the presence of less than the optimal moisture control of the cavity.

These new materials consist of a monomer part and a powder part.

The monomer part (UP monomer- name given due to the presence of UDMA and PPGDMA) of all formulations includes 72% UDMA (Urethane dimethacrylate), 24% PPGDMA (Poly-(propylene glycol) dimethacrylate), 3% 4-META (4-Methacryloxyethyl trimellitate anhydride) and 1% of the initiator CQ (Camphoroquinone). The UP monomer has been described previously (Walters, 2015).

▪ UDMA (Urethane Dimethacrylate)- $C_{23}H_{38}N_2O_8$

UDMA or urethane dimethacrylate is the base monomer of the new formulations. UDMA is a high molecular weight base monomer that has been widely used in dental composites, frequently in combination with Bis-GMA or other similar high viscosity monomers. The use of UDMA has been shown to result in significantly higher conversion, more complete cross-linking and lower leaching of uncured monomer than Bis-GMA (Floyd, 2006) as well as higher flexural strength and hardness. Recent studies have shown that UDMA impacts indeed on the monomer conversion, BFS and depth of cure, leading to better results compared to Bis-GMA- containing composites, without though any increase in shrinkage. Also in some studies UDMA has been found to be less cytotoxic compared to Bis-GMA (Walters, 2015).

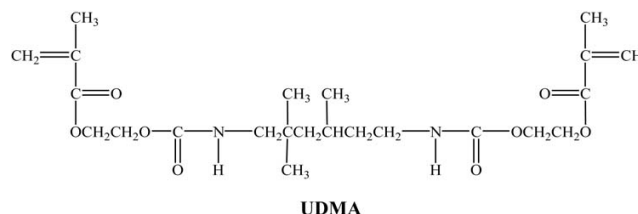


Figure 2.1 Chemical structure of UDMA

- **PPGDMA (Poly-(propylene glycol) dimethacrylate)-** $\text{H}_2\text{C}=(\text{CH}_3)\text{CO} (\text{OC}_3\text{H}_6)_n \text{O}_2\text{CC} (\text{CH}_3)=\text{CH}_2$

PPGDMA or Poly-(propylene glycol) dimethacrylate is a high molecular weight monomer that can be a replacement for TEGDMA. Recent study has shown that due to its high molecular weight and flexibility, a greater monomer conversion can be achieved, thus the amine activator dimethyl- p- toluidine (DMPT) usually needed for the TEGDMA- containing composites can be omitted. PPGDMA has significantly lower double bond concentration compared to TEGDMA and better biocompatibility. It can also increase the depth of cure without affecting the shrinkage (Walters 2015, Ben Nuba 2014).

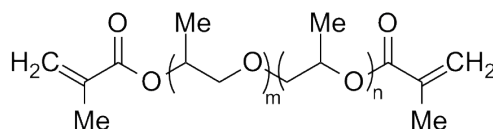


Figure 2.2 Chemical structure of PPGDMA

- **4-META (4- Methacryloxyethyl trimellitate anhydride)-** $\text{C}_{15}\text{H}_{12}\text{O}_7$

4-META or 4-Methacryloxyethyl trimellitate anhydride has been used in the experimental composites of this project as an adhesion-promoting component and is one of the first acidic monomers, widely used in self-etching systems (Chang, 2002). 4-META is a crystalline powder, which, in the presence of water can form 4-MET following a hydrolysis reaction. 4-META has a hydrophilic group ($-\text{COO}-$) that absorbs the water and enables polymerisation and also a hydrophobic phenol and alkyl groups. (Unemori, 2003) It has been shown that 4-MET is able to create an ionic bond with calcium in hydroxyapatite (Yoshida, 2004).

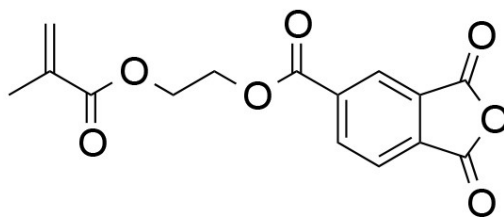


Figure 2.3 Chemical structure of 4-META

- **CQ (Camphoroquinone) – $C_{10}H_{14}O_2$**

CQ or camphoroquinone is a photo-initiator (Stansbury, 2000). The CQ initiates the polymerization process in the presence of a light source. CQ is a commonly found photo-initiator with a tertiary amine as co-initiator in order to start the polymerization process (Maciel et al., 2018). When CQ absorbs light in wavelength between 430 and 510nm (blue region of visible light spectra) with a maximum absorption wavelength at 468 nm, then a photo-excitation complex with a tertiary amine is created and two free radicals are generated. These radicals are amino and cetyl. This is considered a hydrogen abstraction photo-initiation (Asmusen, 2009).

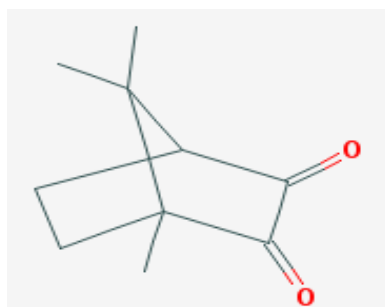


Figure 2.4 Chemical structure of Camphoroquinone (CQ)- $C_{10}H_{14}O_2$ (PubChem)

2.2.2 Fillers of the new formulations (A6, C2, SU2)

The powder part of the A6 formulation consists of hybrid glass (glass 7 μ m, glass 0.7 μ m and nano-glass) as well as **5% MCPM** (monocalcium phosphate monohydrate) and **5% PLS** (polylysine).

The powder part of the C2 formulation consists of similar amounts of hybrid glass but higher amount of **MCPM (10%)** and less than half the amount of **PLS (2%)**.

▪ **MCPM (Monocalcium Phosphate Monohydrate)**

MCPM or monocalcium phosphate monohydrate is a reactive calcium phosphate filler, which is hydrophilic and soluble. Its hydrophilic nature enhances the water sorption, which in return, enables the release of calcium phosphate and phosphoric acid. MCPM also promotes brushite formation, leading to re-mineralisation of the dentine. So MCPM is adding remineralising and self-etching properties to the composite formulations.

▪ **3-Poly-L-lysine (PLS)**

3-Poly-L-lysine (PLS) is an antimicrobial agent, which has been used as a food preservative and has been approved by Food and Drug Administration (FDA). It is effective against a wide range of bacteria and is a biocompatible component displaying low toxicity is produced from fermentation of aerobic bacteria by a non-pathogenic microorganism known as *Streptomyces albulus* (Shih, 2006).

Studies have shown early release of PLS from hydrophilic dental composites and a positive effect on the formation of apatite in CaP composites (Panpisut, 2016). Polylysine is a homo-polypeptide molecule that has residues of L-lysine estimated as 25-30 (Yoshida and Nagasawa, 2003, Hiraki et al., 2003). A peptide bond links the carboxyl and the e-amino group.

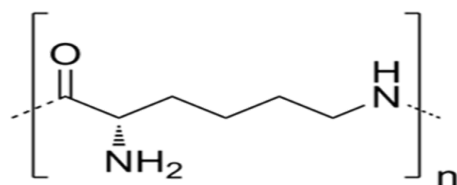


Figure 2.5 Chemical structure of polylysine (PLS)

E-polylysine produced via the gram positive bacteria is about 4700 and the lysine molecules are linked by the epsilon. E- polylysine molecules are cationic with positively charged amine groups in water (Hiraki et al., 2003). Polylysine, has very high water solubility and is biodegradable (Shih et al., 2006). Its use has been advocated in several fields, including food industry, medicine and electronics. The antimicrobial properties it is used as widely as a preservative agent (Shih et al., 2006). Additionally, it is unscented and tasteless making it an ideal preservative. In Japan, it is widely used as a preservative for food such as meat, sushi and rice, in relatively low concentrations (Ye et al., 2013).

Polylysine is known to have broad-spectrum antimicrobial properties. It can impede the growth of a wide range of microbes including yeast, bacteria both gram positive and gram negative (Yoshida and Nagasawa, 2003). The antimicrobial action is believed to be through adsorption into the cell membrane, resulting in abnormalities in the cytoplasm (Najjar et al., 2009).

The powder to monomer mass ratio for all experimental formulations (A6, C2 and SU2) was 3:1 (PLR 3).

The powder-liquid ratio is important for the composites as it determines the hardness or the ability of the material to flow. Both formulations had a PLR 3, as previous research has shown that with PLR 3 the bonding was improved compared to formulations with PLR 5.

Monomer UP		
UDMA	10.800g	72%
PPGDMA	3.600g	24%
4META	0.450g	3%
CQ	0.150g	1%
	15g	

Table 2.1 Composition of UP monomer

A6- filler			C2- filler	
Glass 7	4.860 g	54%	4.752 g	53%
Glass 0.7	2.430 g	27%	2.376 g	26%
Glass nano	0.810 g	9%	0.792 g	9%
MCPM	0.450 g	5%	0.900 g	10%
PLS	0.450 g	5%	0.180 g	2%
	9 g		9 g	

Table 2.2 Composition of A6 and C2 filler part

As shown in table 2.2, the A6 formulation consists of equal amounts of MCPM (5%) and polylysine (PLS 5%), while the C2 formulation contains double the amount of MCPM (10%) and less than half the amount of PLS (2%).

<u>UDMA</u>	High molecular weight base monomer
<u>PPGDMA</u>	High molecular weight diluent monomer Important for flow and handling Improves conversion
<u>4-META</u>	Acidic monomer, adds self-adhesive properties to the formulations
<u>CQ</u>	Camphoroquinone, initiator→ starts polymerisation process

Table 2.3 Monomer part of the formulations A6 & C2, containing UDMA, PPGDMA, 4-META and CQ

<u>MCPM</u>	Reactive calcium phosphate filler Hydrophilic and soluble → enhances water sorption, calcium phosphate release and phosphoric acid release
<u>PLS</u>	Polylysine→ Antibacterial agent, effective against wide range of bacteria
<u>PLR 3</u>	Powder-liquid ratio, important for the flow & handling of the material

Table 2.4 Powder part of A6 and C2 formulations containing MCPM and PLS in a powder: liquid ratio 3:1 (PLR 3)

The tables 2.3 and 2.4 are summarizing the features of each one of the components of A6 and C2 formulations. **Table 2.3** described the monomer part consisting of UDMA, PPGDMA, 4-META and CQ. **Table 2.4** summarizes the powder part, which includes MCPM and Polylysine.

2.2.3 Final formulation – SU2

Following experiments in the laboratory using different formulations, a final version was developed, considered to combine all the desired properties. The SU2 formulation is the final version of the SMART experimental formulations and its composition can be seen in Table 2.5.

The glass filler part of this formulation is similar to the two materials tested before for the shear bond strength (A6, C2), which have been previously described, so the difference comes again in the amount of MCPM and PLS.

The filler of the SU2 formulation comes in 6:3:1 ratio, which is a combination of 7µm glass, 0.7 µm glass and nano-silica glass.

The powder part of the SU2 formulation contains **8 w% MCPM** and **4 w% PLS** (polylysine).

The powder to liquid mass ratio (PLR) was again 3:1.

SU2 composition		
	UDMA	72%
	PPG-DMA	24%
	4-META	3%
	CQ	1%
	MCPM	8%
	PLS	4%

Table 2.5 The composition of the final SU2 formulation (powder and monomer, PLR 3:1)

2.2.3.1 Preparation of the A6 & C2 composite formulations

The composite formulations A6 and C2 were developed by mixing the glass fillers presented in table 2.2 with the monomer part, which can be seen in table 2.1, using a planar mixer at 3500 rpm for 40 seconds. The planar mixer was Speed-mixer in Synergy Devices Ltd. (Speed-Mixer, DAC600.2 CM51, Synergy Devices Ltd, UK)

The scale up process for the SU2 was also carried out using the Speed-mixer DAC600.2 CM51 in Synergy Devices Ltd.

The mixing procedures for the SU2 formulation are as follows:

1. Powder (225g) mixing at 1000 rpm for 30s (no Vacuum)
2. Add monomers (75g)
3. Wetting powder at 2300 rpm for 15s (no Vacuum)
4. Vacuum for 3 mins (no speed), the pressure down to around 20 mbar
5. Mixing at 1800 rpm for 30s (17 mbar)

2.3 Methods

2.3.1 Part 1: Protocol for the preparation of the samples

2.3.1.1 Collection of the teeth

The human teeth used were collected from the Eastman Bio-bank (Reference Number 1304) following consent of the patients. Sound adult molars and premolars extracted less than 6 months prior to the tests were used for the shear bond tests in human dentine. The teeth employed for the first shear bond tests were initially stored into thymol 4% for 24 hours and then kept into distilled water until use.

The protocol changed after the first shear bond tests, so when these were subsequently repeated with new teeth, these teeth were kept in chloramine T 1% for 24 hours and then in distilled water until use to maintain moisture.

Technical specification:

The tests were done following the protocol of the shear bond tests of

ISO/TS 11405

Dental materials- Testing of adhesion to tooth structure

Teeth

According to the technical specification of ISO guidelines, teeth used for the tests should be caries free, or with small restorations away from the adhesion area, and the time after the extraction should be no more than six months. Teeth should be stored in distilled water in a refrigerator. Before doing the tests, the tooth surfaces should be prepared with abrasive paper of a grit size P600 under running water in order to expose the dentine surface.

Ivory dentine

In an attempt to have more repeatable results, samples from a single elephant tusk of ivory dentine were used and tested with an aim to be compared the results with those of the shear bond tests in human dentine.

With the term ivory dentine we define the dentine of any large tusk (Locke, 2008). Ivory dentine can be considered as a composite with inorganic and organic element, containing hydroxyapatite (HA)- like mineralised component (60–70 weight %) in which about 20 % of the Ca^{2+} ions are replaced by Mg^{2+} , embedded within an organic matrix (type I collagen, 30 weight %). Most of the remaining content is water (Jakubinek et al., 2006).

The ivory dentine is composed of a matrix of particles with diameter 5–20 μm , in a ground substance containing dentinal tubules with a diameter of about 5 μm and a center-to-center spacing of 10–20 μm . Dentinal tubules may be straight or curly.

Ivory tubules have similar shape and size with those of human dentine, but their density is lower (Liaqat et al, 2015). In human dentine tubule density varies, with an increased number of them as we go near the pulp.

The structure and composition of the ivory varies in different mammals according to their mechanical necessities. Ivory, by description, is the dentine of large teeth, but teeth became large in response to many different evolutionary stimuli (Locke et al, 2008).

2.3.2 Preparation of human teeth samples

Sound adult molars and premolars have been used from the Eastman Bio-bank after patients were consented and were prepared following the ISO guidelines, as previously described. After the extraction the teeth were cleaned from any remaining human tissues and kept initially in thymol solution 0.1% for disinfection and later in chloramine T 1% for one day for disinfection and in distilled water until use after that.

The preparation of the samples was initiated by embedding the teeth into self-curing resin blocks. As seen in Figure 2.6, the dentine surface of the human teeth was exposed by sectioning the embedded tooth in a direction parallel to the occlusal surface under a constant flow of water with the use of a universal cutting machine (Leica).

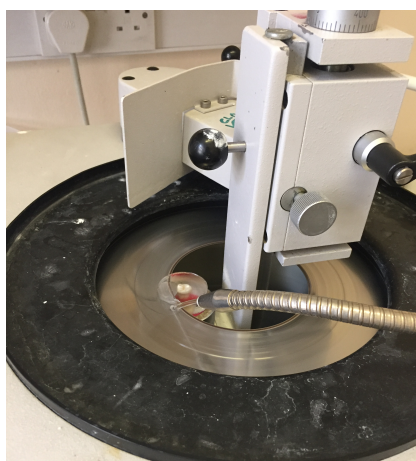


Figure 2.6 Sample embedded in self-curing resin blocks and sectioned with universal machine (Leica) to expose a flat dentine surface

After the cut, the dentine surface was polished with P500 grit silicone carbide paper discs (Struers, Denmark), in order to create the standardised smear layer necessary for the bonding procedure. Following the preparation of the tooth surface, the teeth were stored in water until use to avoid drying of the dentine.

Figure 2.7 shows the samples of the teeth that were embedded in the resin blocks after the dentine surface had been exposed and polished. The pipette cylinders can be seen as well. These cylinders were sectioned in smaller parts of approximately 4mm length and were used as a standardised holder for the placement of each material in the dentine surface.

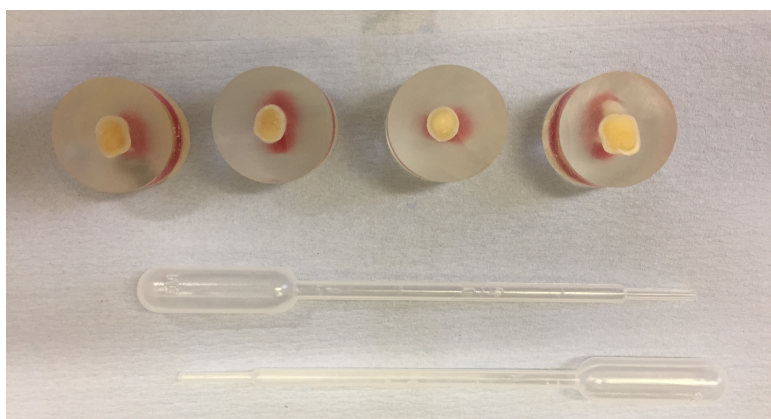


Figure 2.7 Resin blocks containing the human teeth after the dentine has been exposed and pipette cylinders for the application of each material on the dentine surface

Before the application of the composite formulations, the teeth were blot-dried with filter paper for five seconds. The paste was placed in the dentine surface with the help of the plastic pipette cylinders, which had a total area of 10.75 mm^2 and about 4mm height (See Figure 2.7). The composite paste was then light-cured for 40 seconds and the plastic of the pipette was cut with a blade and carefully removed, leaving the composite cylinder in the dentine surface. The final samples as prepared for the shear bond tests are displayed in figures 2.8 a, b and c.

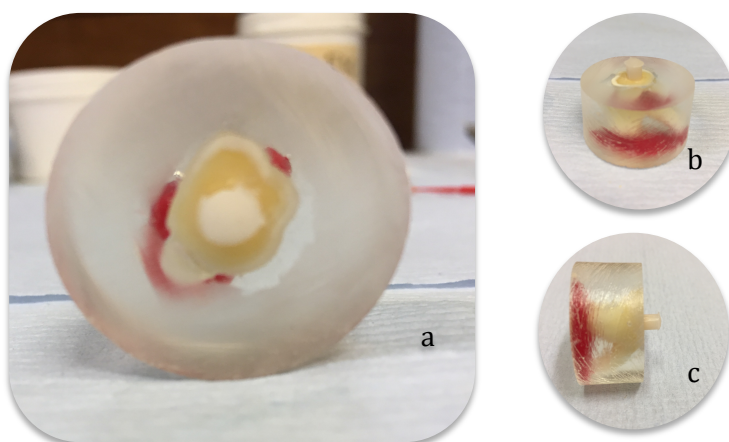


Figure 2.8a, 2.8b, 2.8c Images of the prepared samples of the teeth with the A6 & C2 formulations as they were placed in the dentine surface before the shear bond testing (here without the use of etch or i-bond)

When the material to be tested had been placed in the dentine surface, the samples were ready and were immediately stored in water and kept in an incubator at 37°C for 24 hours. After the 24-hour storage, the shear bond strength of the samples was assessed using a universal machine, the INSTRON 4505.

2.3.3 Ivory samples

The ivory samples were prepared in a similar way as the human dentine samples. The ivory was also embedded in resin blocks and the blocks were kept in water until use to maintain ivory moisture. The material- dentine samples with ivory were prepared following the same process described in the previous section. The ivory surface was gently blot- dried using filter paper and the material was placed using the plastic pipette cylinder with 4mm height and light-cured. The ivory samples were tested in two different temperatures. Some of them were tested after storage in water and in the 37⁰ incubator and some of them were stored for 24 hours in water as well but in room temperature (23⁰).

2.3.4 Part 2: Shear bond tests

For the shear bond tests the INSTRON 4505 universal machine was employed (Figure 2.9). The human dentine and the ivory dentine resin blocks were held in place with a “flat-edge shear fixture jig”. The metal jig has a holder part that keeps the resin block stable and a blade with a flat and sharp edge placed parallel to the dentine surface and against the composite cylinder (see Figure 2.10).

The blade is getting the force from the crosshead and through the blade this force is applied on the material cylinder until the bond between material and dentine breaks.

The crosshead speed of the machine was 0.5mm/min using 1 kN load cell.

The equation used for the calculation of bond strength is:

$$S = \frac{P}{A}$$

P → is the maximum load at the bond breakage

A → is the composite surface area in contact with the dentine



Figure 2.9 INSTRON 4505 Universal Machine for shear bond strength

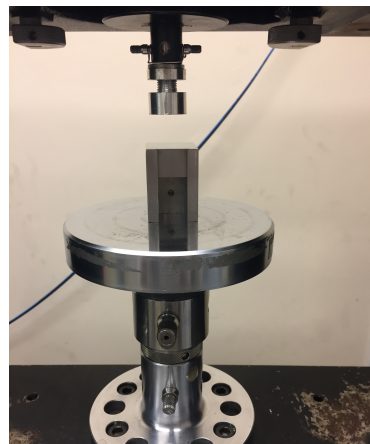


Figure 2.10 Sample holder and jig, INSTRON 4505

Figures 2.9 and 2.10 display the INSTRON machine and the jig that was holding the samples prior to the tests.

Multiple repetitions of the shear bond tests were done for the experimental formulations (A6, C2) and the control materials (Z250, Fuji II-LC, Fuji IX). The first shear bond tests were done without the use of etch & i-bond prior to the application of each material on the dentine surface. That was done to assess the performance of the new materials, which aim to omit the need for using etch and bond.

Later, tests were done using different combinations of etch and bond and different treatments of the dentine prior to the material placement, with an aim to assess how these treatments can affect the bond strength for the new materials.

The two different types of dentine (human or ivory), the combinations of etch and/ or i-bond as well as the dentine surface treatments, are outlined in the table below (Table 2.6):

Shear Bond Tests

1. Without etch or i-bond (A6, C2, monomer UP, Z250, Fuji II, Fuji IX)
2. With etch only→ 20" of etching, followed by rinsing with water, gentle drying and application of the composite (A6, C2, Z250)
3. With i-bond only according to the manufacturer's instructions→ 20 seconds of light curing and application of the composite (A6, C2, Z250)
4. With etch & i-bond
5. Without etch or i-bond but with air-drying the dentin for 60 seconds (A6, Z250)
6. Without i-bond, but with conditioning of the dentine with formic acid for 4 hours prior to the material placement (A6)
7. Using ivory dentine without etch and i-bond in room temperature (C2)
8. Using ivory dentine without etch and i-bond in 37° incubator for 24 hours (C2)
9. Using i-bond to cover the dentin around the formulation (A6)

Table 2.6 Different combinations of etch and/ or i-bond, different types of dentine (human/ ivory) and different treatments of dentine surface before the shear bond tests

The materials that were tested for the first shear bond strength were:

C2 formulation (**10% MCPM, 2% PLS**), **A6** formulation (**5% MCPM, 5% PLS**) and the UP monomer alone, and they were compared to the commercials Z250 (3M) (with and without etch and/ or i-bond) and Fuji II-LC and Fuji IX glass- ionomers.

All the samples tested in both human and ivory dentine, were kept in water and stored in a 37° incubator for 24 hours. Only one round of tests using ivory dentine was done with the samples kept in water but in room temperature (23°) for 24 hours prior to testing.

2.3.4.1 Shear bond tests without etch & i-bond

In the first round of tests ten sound adult molars were used and prepared as described above. Each one of the different materials was placed in the exposed dentine without the prior application of etch and i-bond. The materials were randomly allocated in each tooth and no record of which tooth was used for each material testing was kept. In order to do the multiple repetitions, the teeth were polished after every test with 500 grit polishing discs and were used again. When the pulp was reached, the teeth had to be discarded. The shear bond strength of the A6 and C2 formulations was compared to the shear bond strength of the UP monomer only, as well as to the commercial Z250, Fuji II and Fuji IX, which were used as controls.

2.3.4.2 Problems following first tests

After the first round of tests, the different formulations that were tested presented with great variation in the shear bond results, high standard error and very low shear bond strength for all the formulations apart from Fuji II & Fuji IX.

Some of the teeth used in the first round of tests would not keep any of the materials in place, so a question came up, regarding the differences in the results among different teeth and whether the bonding to human dentine could be tooth-related as well. It was therefore decided to proceed with a second round of tests, using the same tooth for each material to be tested and compare the results among the different materials and the different teeth.

2.3.4.3 Shear bond tests using tooth ID - no etch & i-bond

This time, a number was used to identify each tooth that was prepared as a sample. The same tooth was polished with 500 grit polishing disc and was used again to assess the shear bond strength of the two new formulations (A6 & C2) and the SBS of the commercial control (Z250, 3M).

Seven teeth were used for these tests and the samples were prepared following the same process described before.

2.3.4.4 Shear bond tests with etch or i-bond only or etch & i-bond together

The first tests were done with an aim to assess the shear bond strength of the experimental composite materials without the prior use of etch & i-bond as these new materials are developed to reduce the steps of the composite application and completely exclude the need for prior use of etch and bonding agents.

Following the above tests, the shear bond strength measurements were repeated for each formulation (A6, C2), this time to determine how the different conditioning of the dentine and the additional application of etch and bond would affect not only the shear bond strength, but also the consistency of the results, which presented with great variation during the previous tests.

The shear bond strength of the two new formulations (A6, C2) was first tested with application of i-bond only before the application of the material. The samples were prepared as described previously, but this time the dentine surface was blot-dried and i-bond (total etch system, iBOND^R Total Etch) was applied according to the manufacturer's instructions and light- cured for 20 seconds. Then, each material was placed with the pipette cylinder and light- cured for 40 seconds. The commercial Z250 was used as control in these tests and was also placed in the dentine surface with i-bond only.

2.3.4.5 A6 formulation tested using etch – no i-bond

The shear bond strength of the A6 formulation was assessed following conditioning of the dentine of seven teeth with 30% phosphoric acid (total etch system, 3M). The etch was applied in the dentine for 15 seconds according to the manufacturer's instructions, with an aim to demineralise the surface, expose the collagen fibrils and assess if etching the dentine has positive or negative effect on the bond strength and on the performance of the material. After the conditioning, the material was placed in the dentine with the same pipette cylinder as described in the previous tests.

2.3.4.6 A6 formulation tested with etch & ibond

Five samples were prepared as described in the previous tests. The dentine surface was conditioned again with 30% phosphoric acid for 15 seconds and then i-bond was applied and light-cured for 20 seconds (total etch system, iBOND^R Total Etch) according to the manufacturer's instructions. The A6 formulation was subsequently placed using the pipette cylinder. The commercial Z250 was placed with etch and iBond as well and was used as control.

2.3.4.7 Air-dried teeth for 60'' compared to Z250 with etch & i-bond

After completing the tests with etch and iBond, the experimental formulations A6 and C2 were tested again without etch and iBond but the dentine was now over-dried with air for 1 minute (60'' air-dried dentine) instead of being blot- dried with filter paper as in previous tests.

In this round of tests the commercial composite Z250 was again the control and was tested in the same teeth, but this time the Z250 was applied as per manufacturer's instruction with prior use of etch and i-bond.

2.3.4.8 Demineralised model- Use of formic acid

Following the previous tests, teeth were collected and prepared as analysed before exposing the dentine surface. Subsequently they were stored in formic acid for four hours. That test was done with an aim to expose a collagen surface and attempt to mimic the carious lesion. As mentioned previously, the aim of the experimental formulations is to have adequate bonding even when caries remains in the cavity and also remineralise the lesion.

The impact of it on the shear bond strength was assessed with the samples as previously. The A6 formulation was tested in the teeth that had been demineralised. The results can be seen in table 3.6 and in graph 3.6.

2.3.5 Scanned Electron Microscopy (SEM) to determine the dentine surface prior and after SBS tests

The scanning electron microscope (SEM) is a high magnification microscope. A source of electrons, a column for the electrons to move through, an electron detector, a vacuum chamber for the sample and a computer are the main elements of an SEM.

The SEM can assess the interface between materials and create an image of it with the use of a high-energy beam of electrons. As the electrons interact with the sample, they produce secondary electrons, backscattered electrons and characteristic X-rays. One or more detectors collect these signals in order to form images, which are then displayed on the computer screen. When the electron beam hits the surface of the sample, it penetrates the sample to a depth of a few microns, depending on the accelerating voltage and the density of the sample. Many signals, like secondary electrons and X-rays, are produced as a result of this interaction inside the sample. Figure 2.11 is describing how the SEM works and displays its different parts.

The SEM was employed in this part of the project in order to assess the morphology of the dentine and dentinal tubules of the teeth that were tested with the new composite formulations and compare them to sound teeth that had not been tested before. That was done with an aim to understand how the dentine looks prior and after the shear bond tests and if there are any characteristics that would affect the bonding of the materials tested prior and after the shear bond tests.

The SEM was also used in the following parts of the project in order to assess the presence of resin tags in the material/ dentine interface when the SU2 formulation was used in cavities of carious primary molars without caries removal or with partial caries removal.

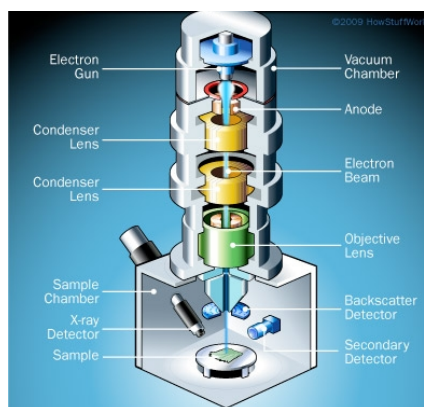


Figure 2.11 SEM and how it works (Image taken from <https://science.howstuffworks.com/scanning-electron-microscope2.htm>)

For the assessment of the dentine of sound teeth before the shear bond tests, the teeth were embedded into self-curing resin blocks as it was previously described and were cut using the same universal machine blade (Leica machine) to expose the dentine surface. The sections were done either in the occlusal plane with a horizontal section parallel to the occlusal surface, or vertically in order to assess the dentine on the buccal surfaces of the teeth.

The tested teeth were scanned following the shear bond tests, without polishing or conditioning of the tooth surface. Consequently some parts of the experimental composites remained in the dentine surface and can be seen in the scans. (Figures 3.3, 3.5, 3.6, 3.7)

Before the use of the SEM, all teeth were sputter coated with gold alloy.

2.3.6 Actions further to previous outcomes

Following the previous rounds of tests, it was found that there was a great variation in the results for all the teeth and the different dentine treatments. It was therefore decided to repeat some of the tests using ivory dentine. The ivory dentine has been found to have similar features with human dentine, but since it comes as a big block, as previously analysed, it has more consistency in its structure and there is no pulp and no variation in the density of the dentinal tubules as it happens with human dentine. As a result, the shear bond tests can be more repeatable and the sample size can be bigger.

2.3.6.1 Tests with ivory dentine

The experimental formulation C2 was tested in ivory dentine as well. The aim was to make comparisons to the results after the tests using human dentine and to assess the differences in shear bond strength between the two dentine types and also assess the consistency of the results. The material (C2) was tested in two different temperatures; twelve samples were prepared and tested in room temperature and eight samples were kept in the 37° incubator as the samples for all the previous tests.

These tests were aiming to determine whether there is an effect of the temperature on the bond strength of the material and how the ivory dentine and/ or the temperature would affect the consistency of the results compared to the human dentine. The commercial Z250 was also tested with ivory and was the control. For all materials no etch or i-bond was used prior to the application in ivory dentine.

2.3.7 Part 3: Sealing ability and resin tag formation

This part of the research had as main goal to assess the sealing ability of the last version of formulations, which is the SU2 (composition described in table 2.5) and the ability to create resin tags when no caries is removed from the cavity (infected dentine) or when caries is partially removed from the cavity (affected dentine).

The matters that had to be investigated are as follows:

- Has the new composite formulation adequate sealing ability?
- How much caries needs to be removed before placing the material in order to achieve the best resin tag formation?
- Are there any resin tags created in the interface between the cavity and the material when no caries removal or when selective caries removal takes place?
- How does no caries removal affect the presence of the resin tags?
- How dry/wet should the cavity be, in order to achieve the best sealing / resin tag formation?
-

In order to assess the sealing ability and resin-tag formation of the final formulation (SU2), carious primary molars were collected from the Eastman Biobank.

More specifically, carious primary molars were collected and excavated to give two groups with either minimal or partial caries removal. For each group, equal number of teeth was filled with the experimental composite SU2 and with Fuji II LC. Half of the teeth that were filled with SU2 had no caries removal and half had partial caries removal and the same happened with the teeth that were filled with Fuji II- LC.

Following the above process, the teeth were kept either in SBF or in Artificial Saliva for 1 week or for 1 month. When they were removed from the solutions, they were embedded in self-curing resin blocks, the dentine / material interfaces were exposed through sectioning and examined qualitatively using Light, Confocal and Scanning Electron Microscopies. The procedures are more thoroughly described for each round of tests in the next sections.

Preparation of SBF

Simulated body fluid (SBF) was prepared in order to store the samples and subsequently assess the formation of hydroxyapatite and resin tags in the interface between the material and the dentine.

The simulated body fluid (SBF) is an inorganic solution whose constitution is similar to that of the human blood plasma, but lacking the organic components of plasma and has the ability to form apatite on the surface.

(ISO- <https://www.iso.org/obp/ui/#iso:std:iso:23317:ed-3:v1:en>)

The SBF was prepared according to the **BS ISO 23317:2012** Implants for surgery – In vitro evaluation for apatite forming ability of implant materials

Figure 2.19 shows the schematic representation of the apparatus that is used in order to prepare the SBF in the lab.

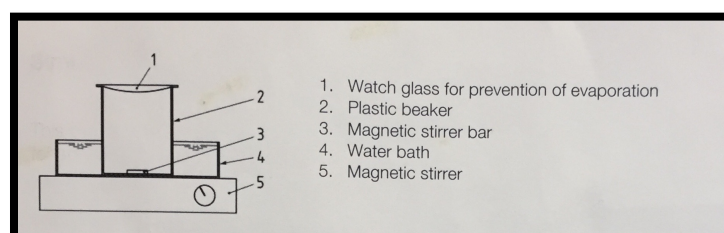


Figure 2.12 Schematic representation of the apparatus for the SBF preparation

The SBF is composed by deionized water, sodium chloride, sodium hydrogen carbonate, potassium chloride, di-potassium hydrogen phosphate trihydrate, magnesium chloride hexahydrate, 1M hydrochloric acid solution, calcium chloride, sodium sulfate and tris- hydroxymethyl aminomethane (TRIS).

2.3.7.1 1st round of resin tag & sealing assessment

For the first round of tests, the teeth selected had Class II cavities of similar size and were divided into two groups. In both groups, no caries was removed from the cavities prior to the placement of the restorative material. These cavities were blot-dried and divided into two sub-groups. The teeth of the first sub-group were filled with SU2 and the teeth of the second sub-group with Fuji II-LC material and were used as control.

Following the above process, the teeth were embedded in self-curing resin blocks. Subsequently, the teeth of the 1st group were placed in SBF and the teeth of the 2nd group were kept in Artificial Saliva (AS) and all samples were stored in a 37° incubator for one week. After this time, the interface between dentine and material was exposed by sectioning the teeth with the use of a universal machine and P500 grit polishing discs. (Struers, Denmark) The interface between material and dentine was assessed with microscope and SEM for the presence of resin tags.

2.3.7.2 2nd round of resin tag & sealing assessment

In this round of tests, four teeth with Class II cavities were collected from the Eastman Biobank and were filled with SU2 material without prior caries removal from the cavities and the samples were prepared as previously described. Figures 2.20 and 2.21 show the carious primary molars before and after placement of the restorative material.

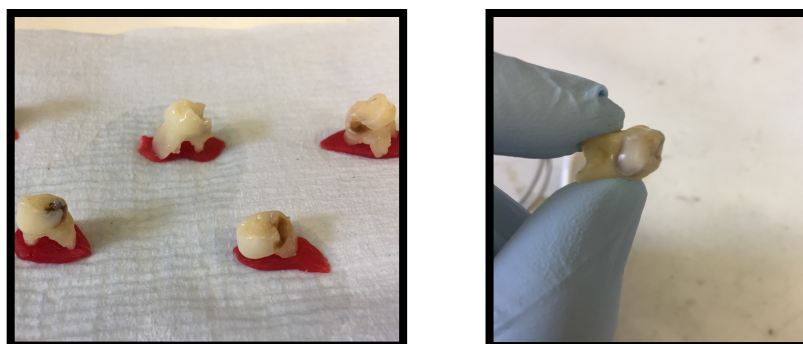


Figure 2.13, Figure 2.14 Primary molars with Class II cavities before and after SU2 placement

Two of these teeth were then placed in SBF and two in Artificial Saliva (AS) and were kept in the 37° incubator, this time for one month in order to assess the resin tag and hydroxyapatite formation. After one month, the samples were removed from the incubator and were then embedded into self-curing resin blocks. The interface between the dentine and the restorative material was exposed again using a polishing disc of P500 grit as described previously. The interface of the teeth kept in SBF solution was analysed for the presence of hydroxyapatite using RAMAN spectroscopy and also microscope and SEM. The results of the RAMAN can be seen in Graph 3.10 and the SEM results are seen in figures 3.12a, b and c.

2.3.7.3 3rd round of resin tag & sealing assessment

For the third round of tests, four carious primary molars with similar cavities were selected and prepared as described above.

2 teeth → no caries removal: one of them filled with SU2 and one with Fuji II- LC

2 teeth → partial caries removal with excavator, one filled with SU2 and one with Fuji II- LC

For the no caries removal group, the teeth were blot-dried before the filling; the material was placed in the cavity and kept in SBF for 7 days into the 37°C incubator. Subsequently, they were embedded in self-curing resin blocks and then ground flat until the dentine was exposed, using a 500 grit polishing disc. Images of the interface between the dentine and the material were then taken first with the light microscope and then using the SEM.

For the partial caries removal group, the soft carious (infected) dentine was removed with the use of an excavator to hard dentine (affected dentine). The teeth were stored in SBF solution and kept in the 37° incubator for 7 days. The samples were prepared as previously described:

- Blot-dried
- Filled with SU2 or Fuji II- LC and light-cured for 40 seconds
- Kept in SBF for 1 week embedded in self-curing resin blocks the interface was exposed using P500 grit polishing discs.

This group was also assessed for resin tag formation and for that reason the Light microscope, the SEM (Scanning Electron Microscopy) and the confocal microscopy were employed.

2.3.7.4 Confocal microscopy for resin tag assessment

Following the assessment with the light microscope and the SEM, this last group of teeth was also assessed using the confocal microscopy.

Confocal microscopy has the ability to control the depth of field, to eliminate or reduce the background information away from the focal plane, which can cause image degradation and the capability to collect serial optical sections from thick specimens. The main principal of the confocal microscopy is the use of spatial filtering techniques to eliminate out-of-focus light or glare in specimens whose thickness exceeds the immediate plane of focus.

The samples were polished in order to remove the gold alloy coating done for the SEM imaging and then they were prepared for scanning with confocal. The samples were dyed using rhodamine b dye prior to scanning and the sample surface was screened with oil objective lens x20 and x60. These samples were assessed again for resin tag formation and the results can be seen in Figures 3.10, 3.11.

2.3.8 Part 4: Assessment of the monomer conversion

The aim of the fourth part of this project was to assess if the final formulation of the new composite (SU2), designed for restoring primary molars, had sufficient monomer conversion in order to achieve single step bulk placement.

The monomer conversions (MC) of the new composite (SU2) were assessed at depth of 2 mm with 20 seconds light exposure versus depth of 4 mm with light exposure for 40 seconds and were subsequently determined using ATR FTIR. A commercial composite (Z250, 3M), a resin modified glass ionomer cement (Fuji II-LC, GC) and a commercial compomer (ACTIVA™ BioActive) were used as controls.

Tree repetitions of the tests were done for each material in each thickness (n=3).

For each one of the four materials a sample for testing was created with the help of a clip ring of 10mm diameter and 1mm thickness. To achieve 2mm and 4mm thicknesses two and four rings were used respectively. The rings were filled with the material to be tested and were then placed over the ATR diamond and covered with an acetate sheet.

Subsequently, the FTIR would run for 20 seconds before the start of curing of each material. The 2mm thickness was cured for 20 seconds and the 4mm thickness for 40 seconds.

Fuji II- LC composition

The RMGIC, Fuji II-LC was used as one of the control materials for assessment the monomer conversion and chemistry. Fuji II- LC liquid consists of 30-40% HEMA (Hydroxyethyl methacrylate), 5-10% TEGDMA (triethylene glycol dimethacrylate) and 20-30% polyacrylic acid. This liquid phase is mixed with powdered alumino-silicate glass.

The GIC, Fuji IX liquid is a 40-50% polyacrylic acid in water solution. This is mixed with a basic glass powder in a powder to liquid mass ratio of 4:1 ratio. This information is according to the Manufacturer's Safety Data sheets (Young et al, 2002).

2.3.8.1 Chemistry of the materials

In order to assess and compare the differences in the chemistry of the four materials, the ATR FTIR was employed and the spectrum differences of the materials were assessed. The SU2 formulation was compared with the Fuji II-LC, Z250 and Activa in wavelength approximately up to 1700 nm.

2.3.8.2 Monomer Conversion

The degree of monomer conversion is important for a resin composite restoration, as it determines and affects its mechanical properties as well as the biocompatibility and colour stability of the material. (Schroeder and Vallo, 2007, Demarco et al., 2012).

Several factors can affect monomer conversion in a composite system, including the monomers used the curing time and the light strength (Lui et al., 2013).

In order to achieve biocompatibility, when a composite material is cured no contents should leach out of it. (Walters et al., 2016; Demarco et al., 2012).

2.3.8.3 ATR FTIR

The computer controlled Attenuated Total Reflectance Fourier transformed infrared (ATR FTIR) was used in order to assess the monomer conversion when the SU2 material was light-cured in two different thicknesses, for 20 seconds in 2mm thickness and for 40 seconds in 4mm thickness.

The spectra of the materials tested using the ATR FTIR can be divided into three important ranges. These are between 3500-2700, 1800-1200 and 1200-700 cm^{-1} .

These ranges have been determined by moisture and resin or resin and inorganic powder phase peaks. The sum of the total absorbance in each range can be used to assess variation between the chemistries of the different materials tested and to ensure quality control. The results require three repetitions and are provided with standard deviations.

Apart from the monomer conversion, the ATR FTIR provides the delay time, the reaction rate ξ and the $t_{0.5}$. The delay time t_d is the time before any reaction of the material at the bottom of the specimen. The reaction rate is the gradient of conversion versus time between 10% and 50% of monomer conversion and the $t_{0.5}$ represents the time for the 50% of the final monomer conversion. They are calculated from the absorbance of at 1319 cm^{-1} minus the baseline absorbance at 1336 cm^{-1} .

The equations used are:

$$P = A_{1319} - A_{1336} \quad M_{\infty} = \frac{P_0 - P_t}{P_0} \times 100$$

Where:

P_0 is peak height before cure

P_t is peak height at time t

M_{∞} is the monomer conversion as time t tends towards infinity obtained from the intercept of later time data plotted versus inverse t .

The reaction rate ξ can be calculated when the slope and intercept of monomer conversion is determined versus time using data between 10 and 50% monomer conversion. Then the ξ is equal to the gradient.

Finally, the delay time t_d is equal to minus the intercept on the y axis divided by the gradient. Then the $t_{0.5}$ can be calculated from the equation:

$$t_{0.5} = \frac{0.5M_{\infty} - t_d}{\xi}$$

Chapter 3

3 Results

3.1 SEM results- Dentine of sound molars and premolars

The dentine of sound teeth was assessed as described previously for characterization of the dentine surface prior and after the SBS tests with an aim to find out if there is anything in the dentine surface that could affect the SBS results. The SEM figures can be seen (Figures 3.1 to 3.7).

In these SEM figures the dentinal tubules are not visible in any of the scans, either with sound or with tested dentine, as we can see in figures 3.3 to 3.6. However, the dentine was not conditioned with etch prior to the scans. The uneven, with no visible dentinal tubules dentine surface seen in the scans can be due to the smear layer created in the dentine surface following the cut, which remained in the surface covering the dentinal tubules. Similarly, the teeth that had already been used for the tests were screened after the shear bond tests. Like the sound teeth, these tested teeth were not prepared prior to testing in order to assess the morphology of dentine as well as the bond between the dentine and the material that had remained in the tooth surface following the tests.

Figure 3.1 shows an occlusal cut of a sound premolar with 2500.0x magnification. The dentinal tubules are not visible and there is a quite rough disturbed dentine surface observed. This is possibly due to the impact of the cutting machine and the polishing disc on the dentine surface.

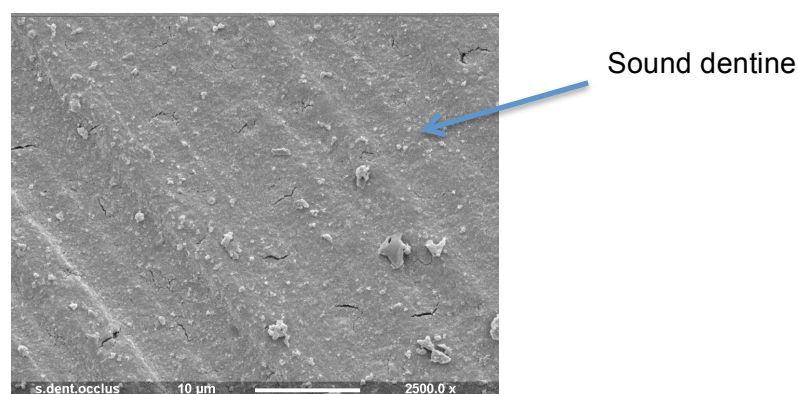


Figure 3.1 Image obtained from SEM microscopy. Sound dentine of the occlusal surface of a premolar in 10µm, 2500.0x magnification

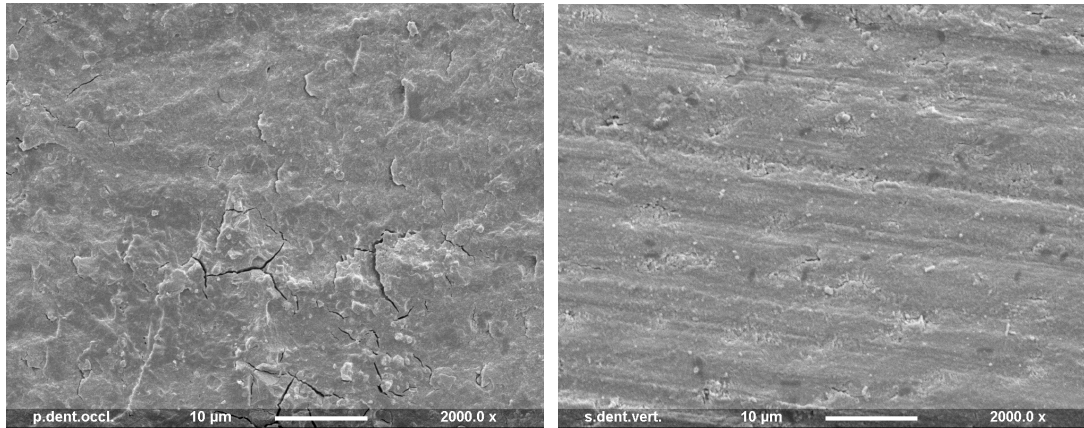


Figure 3.2 Image obtained from SEM microscopy. Dentine of the occlusal surface of molar following testing of composite material, 10µm magnification

Figure 3.3 Image obtained from SEM microscopy following vertical cut of sound premolar in order to expose the dentine surface in 10µm, 2000.0x magnification. No dentinal tubules are visible as no prior conditioning of the dentine was done

Figure 3.2 displays the SEM image of dentine following testing of the tooth for the shear bond strength of the experimental and commercial composite materials.

In figure 3.3 we can see the SEM image of a sound tooth before testing. The first image shows that the dentine surface looks rough and disturbed following the tests. In the second image the dentine surface looks less disturbed, but no dentinal tubules can be observed, possibly because of the lack of previous conditioning of the dentine before the SEM imaging.

3.2 SEM results- Dentine of teeth following shear bond tests

The figures 3.4 and 3.5 present the dentine surface of tested samples and composite material is still seen on the dentine surface following the shear bond tests.

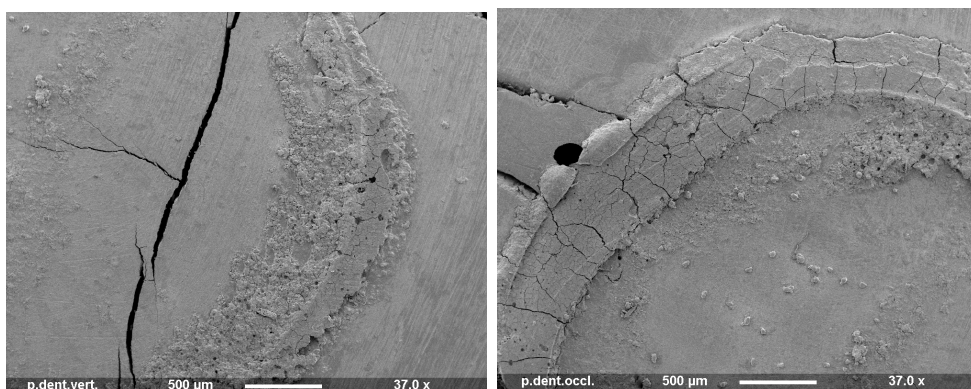


Figure 3.4, Figure 3.5 Figures obtained with SEM microscopy. Dentine surface of samples following shear bond testing with experimental composite remaining in the surface. Both 2.16, 2.17 at 500µm, 37.0x magnification

Figures 3.4 and 3.5 display the dentine of tested teeth, where both dentine and composite material can be seen. The part of the image creating a circle is the material remaining in the dentine following the shear bond tests.

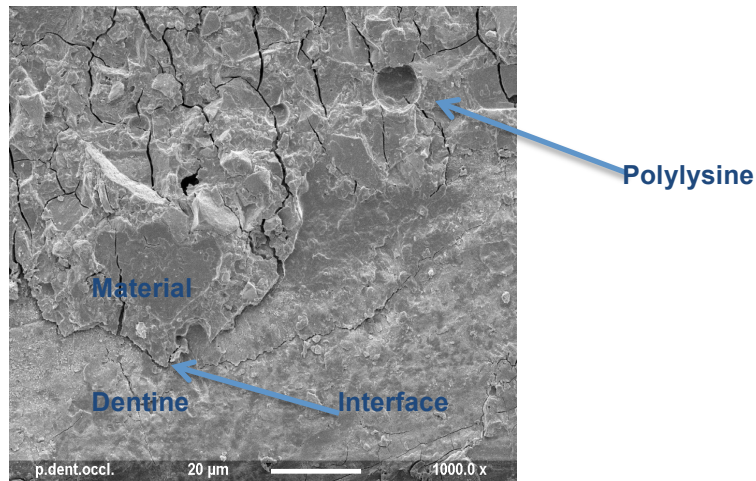


Figure 3.6 Figure obtained with SEM microscopy. Dentine surface of adult molar sample following the shear bond test with the experimental composite formulation, part of which still remains in the tooth surface. Polylysine can be seen. Image 20μm, 1000.0x magnification

In figure 3.6, the material and dentine interface following the shear bond tests can be seen as marked with the arrow.

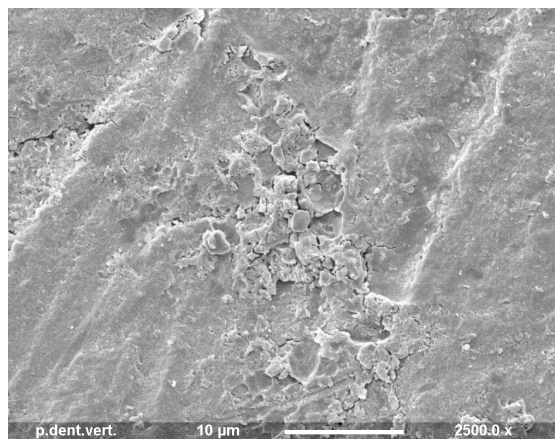


Figure 3.7 Image obtained with SEM microscopy of tested dentine surface, with vertical cut, 10μm, 2500x magnification

Figure 3.7 shows again the dentine surface of a premolar, following shear bond test.

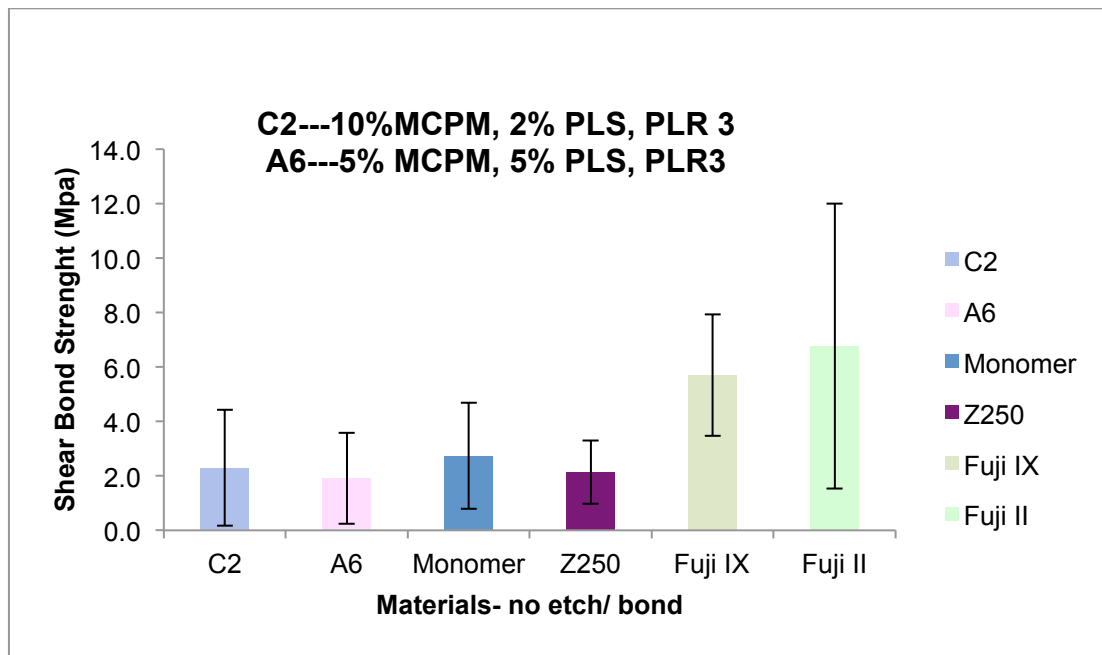
3.3 Results of shear bond strength tests

3.3.1 First round of tests- no use of etch & i-bond

The table 3.1 displays the results of the first shear bond tests, followed by the graph 3.1. Due to the great variability of the results of the shear bond tests, they are presented both in tables and graphs. The mean shear bond strength for the new formulations C2 and A6 without the use of etch and bond is 2.3 Mpa and 1.9 Mpa respectively, which is considered a very low result for the shear bond strength.

	C2	A6	Monomer	Z250	Fuji IX	Fuji II
	0.0114	0.0051	0.0738	0.0171	0.0693	0.0123
	0.0201	0.0349	0.0324	0.012	0.0378	0.0724
	0.0289	0.0095	0.0161	0.0154	0.0706	0.0763
	0.0052	0.0556	0.0335	0.0318	0.0353	0.1623
	0.0177	0.0318	0.0233	0.0475	0.0913	0.1222
	0.0353	0.0029	0.0058	0.0208	0.0285	0.0596
	0.003	0.003	0.0362	0.0165	0.0734	0.0042
	0.0787	0.017	0.014		0.0842	
	0.0496	0.0245				
	0.0172					
	0.0045					
Average	0.02469	0.02048	0.02939	0.02301	0.06131	0.07276
STDV	0.02287	0.01793	0.02088	0.01251	0.02398	0.05628
Av. Mpa	2.3	1.9	2.7	2.1	5.7	6.8
STDV Mpa	2.1	1.7	1.9	1.2	2.2	5.2

Table 3.1 Results of 1st round of shear bond strength tests– (C2, A6, Monomer, Z250, Fuji IX, Fuji II)



Graph 3.1 Results of the 1st round of shear bond tests- no etch or i-bond used

As seen in table 3.1 and graph 3.1, the standard error for this round of tests is relatively high and the results came up with great variation. The highest results were noted in the Fuji II and Fuji IX groups, with means of 6.8 Mpa and 5.7 Mpa respectively. These results although higher, appeared to have high standard errors as well compared to the rest of the materials. The commercial Z250 presented with a mean of 2.1 Mpa SBS.

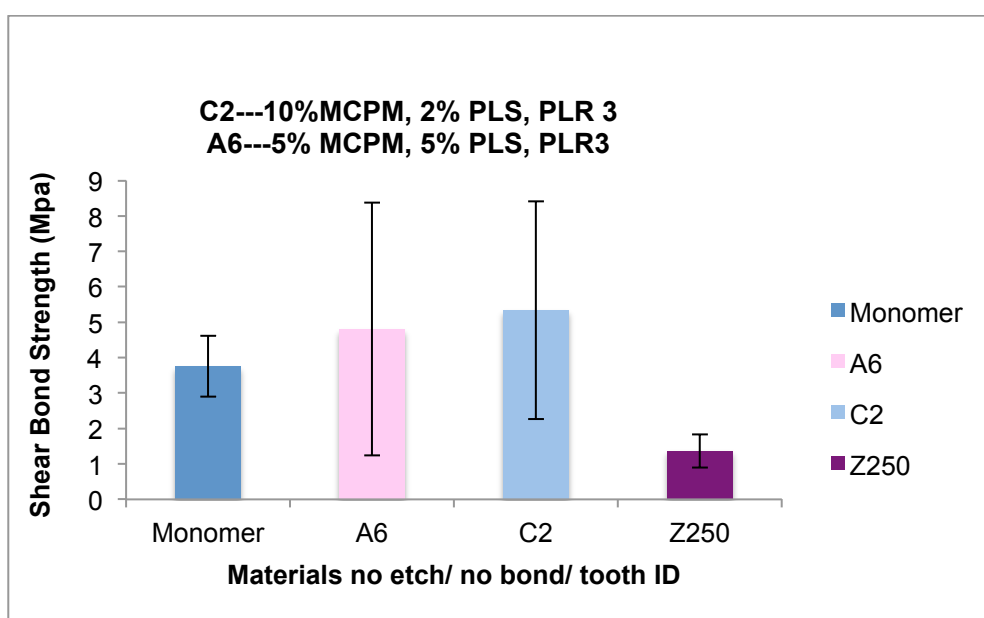
3.3.2 Shear bond test without use of etch & i-bond but with tooth ID

The formulations that have been tested and compared for the shear bond strength in this round of tests were the monomer, the A6, the C2 and the commercial Z250 as control. The results are displayed in table 3.2 and in graph 3.2.

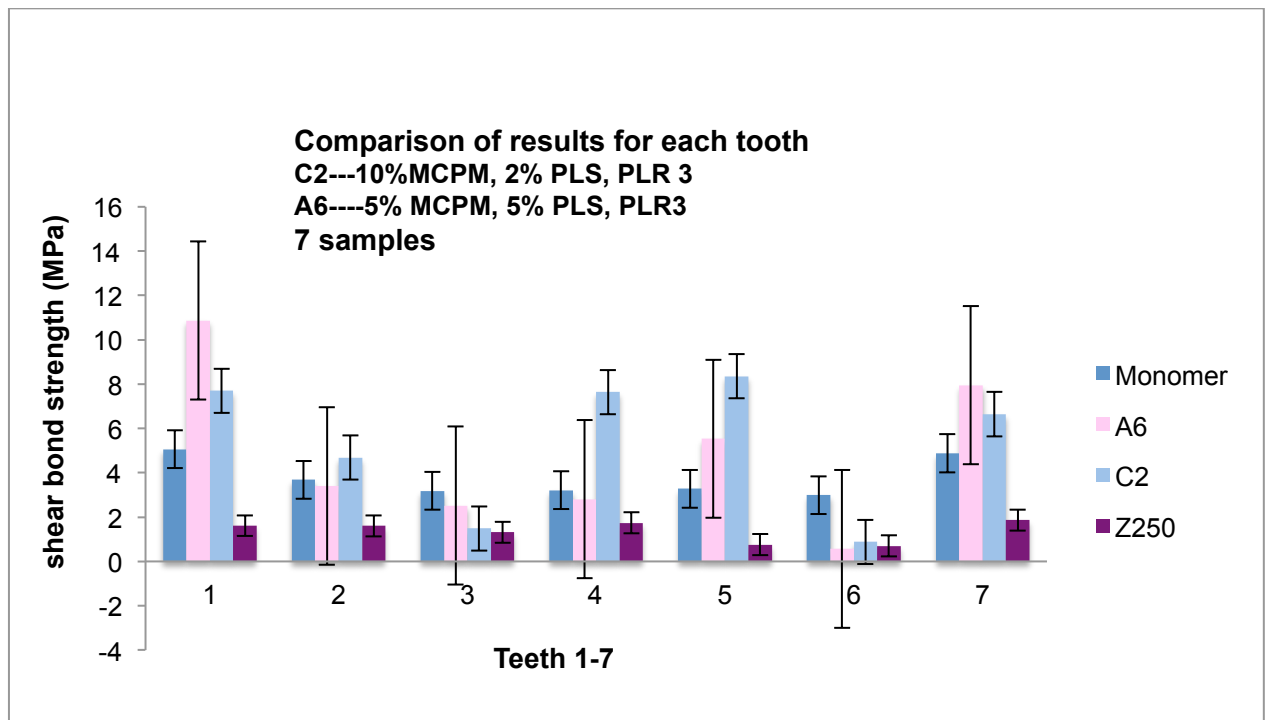
No etch or i-bond on the samples were used again in these tests, however, each tooth was identified by a number and the results in every row of table 3.2 represent a specific tooth, which has been used to test each one of the four materials.

Tooth ID	Monomer	A6	C2	Z250
1	5.1	10.9	7.7	1.6
2	3.7	3.4	4.6	1.6
3	3.2	2.5	1.5	1.3
4	3.2	7.9	7.6	1.7
5	3.3	2.8	8.3	0.8
6	3.0	0.6	0.9	0.7
7	4.9	5.5	6.6	1.9
Average SBS Mpa	3.8	4.8	5.3	1.4
STDV	0.9	3.6	3.1	0.5

Table 3.2 Results shear bond tests of monomer, A6, C2 and Z250 without etch & i-bond



Graph 3.2 Results of 2nd round of tests with tooth ID- Monomer, A6, C2 and Z250 with no etch or i-bond.



Graph 3.3 Results for each tooth tested with the different materials (Teeth 1-7 tested with monomer, A6, C2 and Z250)

These results now show more consistency for all materials apart from A6, which still displays high standard error. Graph 3.2 shows that the C2 had the highest shear bond strength, while the commercial Z250 had a very low SBS when no etch and i-bond was used. The standard error was relatively high.

Specifically, the C2 formulation gave the highest shear bond strength with a mean of 5.34 MPa, followed by the A6 with mean of 4.8 Mpa. The Z250 had very low shear bond strength with mean 1.86 MPa. Furthermore, as seen in each row of the table and when comparing the columns in the graph, specific teeth could display higher or lower shear bond strength measurements for all the different materials tested on them, thus some correlation between the tooth and the result can be seen.

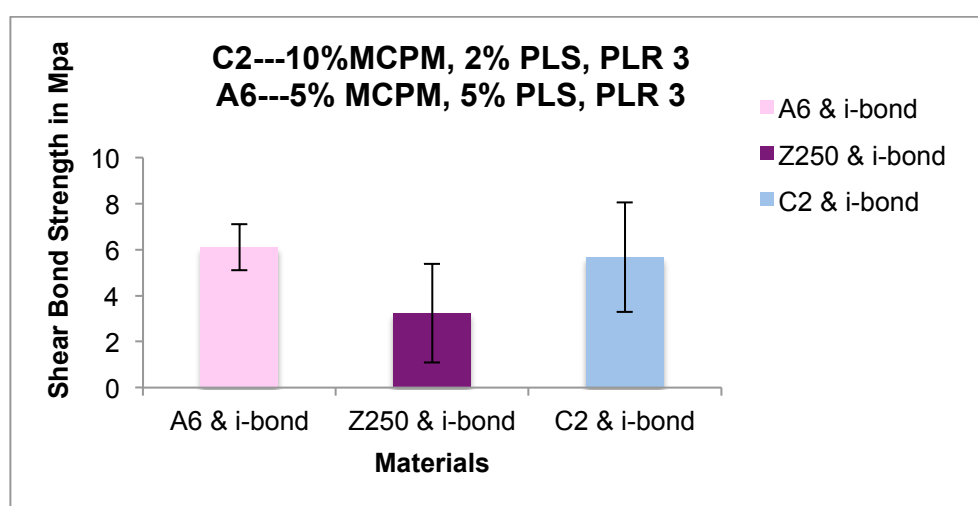
The next results are following the various treatments done in the dentine prior to the material application, such as etch only, i-bond only, combination of etch & i-bond, formic acid and over-dried dentine.

3.3.3 Results of A6, C2 and Z250 with i-bond and no etch

The following results are from the shear bond strength measurements for each formulation, this time using i-bond only. The results of the shear bond tests for A6 and C2 with the use of i-bond only are as seen in Table 3.3. The Z250 was the control material also tested with i-bond only in this round of samples.

SBS Mpa	A6 & i-bond	Z250 & i-bond	C2 & i-bond
	4.9	0.1	3.1
	6.9	4.4	7.4
	6.5	3.8	4.3
		4.7	7.9
Average	6.1	3.2	5.7
STDV	1.1	3.3	3.5

Table 3.3 Results of shear bond strength for A6, C2 & Z250 with i-bond only



Graph 3.4 Graph presentation of shear bond strength results of A6, C2 & Z250 with i-bond only

The Table 3.3 and the Graph 3.4 show that the tests with i-bond only, gave more consistent results, without the great variations that characterised the previous round of tests. The results were still quite low, with a mean of 6.1 MPa for the A6 formulation and 5.7 MPa for the C2. The Z250 has again displayed the lowest result.

The commercial Z250 had always the lowest shear bond strength in all the tests done, even when used with i-bond, with a mean of 3.24 Mpa.

3.3.4 A6 formulation tested using etch only

The A6 formulation was assessed for the shear bond strength following conditioning of the dentine of seven teeth with 30% phosphoric acid for 15 seconds and then rinsed and dried with filter paper before the application of the material. The results can be seen in table 3.4.

SBS Mpa	A6 & etch
	1.8
	5.3
	0.6
	1.7
	0.0
	0.5
	1.2
Average	1.6
STDV	0.4

Table 3.4 Results of shear bond strength of A6 when etch only is used

As seen in the results above, the use of etch only had a negative impact on the bond strength and the formulation had hardly any bond into the tooth surface for all the teeth that were used, with a mean of 1.6 Mpa. Only one sample showed higher result with a 5.3 MPa shear bond strength.

3.3.5 A6 with etch & i-bond

The results of five samples of the A6 formulation with etch & i-bond are presented in the table 3.5. The commercial Z250 was used as control, placed with etch & i-bond as well.

SBS Mpa	A6 & etch + i-bond	Z250 etch & i-bond
	15.2	16.7
	8.0	16.4
	3.3	23.4
	2.6	13.5
	11.5	13.7
Average	8.2	16.8
STDV	2.6	4.0

Table 3.5 Results of shear bond strength of A6 with etch & i-bond

In this round of results, the shear bond strength shows a considerable improvement, something that can be explained by the fact that both etch and i-bond were used, as well as because of the less fine surface created by the use of a more coarse polishing disc.

In this round of tests has shown that the A6 formulation, when used with etch & i-bond, achieved the highest shear bond strength so far with up to 15 Mpa.

The Z250 with etch and i-bond gave the highest results achieved so far with a mean of 16.7 MPa. A combination of all shear bond strength results without etch & bond can be seen in Graph 3.7.

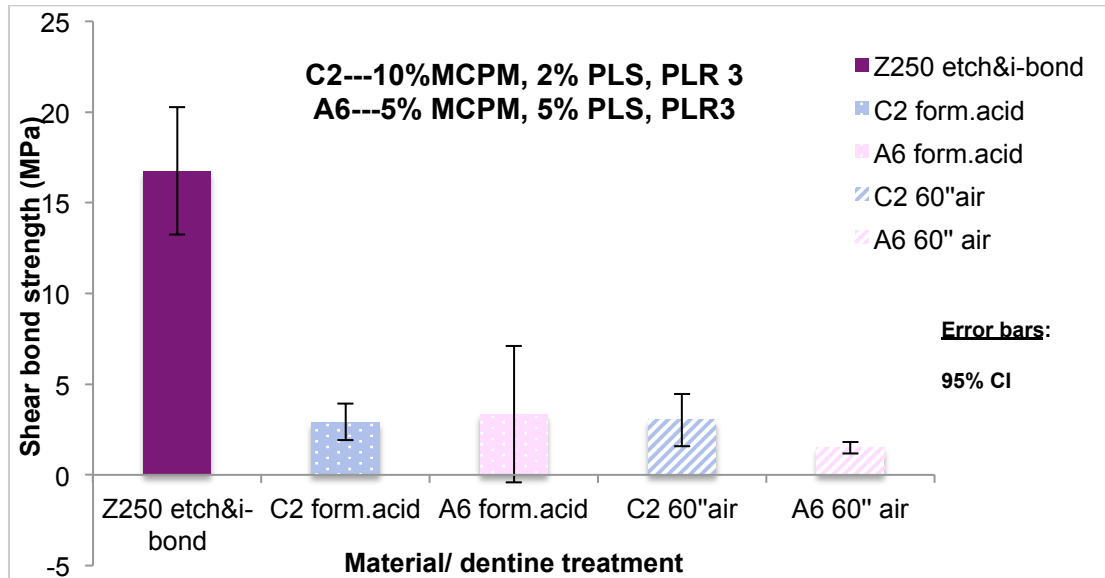
3.3.6 Air-dried teeth for 60s and Z250 with etch & bond

The novel formulations A6 and C2 were tested without etch and i-bond but the dentine was over-dried with air for 1 minute (60s air-dried dentine). In this round of tests the Z250 commercial composite was used as control and was tested in the same teeth but with the additional use of etch and i-bond.

The results are displayed in the table and graph.

SBS Mpa	A6- 60s air	C2-60s air	Z250	C2 form.acid	A6 form.acid
	1.7	2.6	16.7	2.33	
	1.0	5.0	16.4	4.07	
	1.5	1.5	23.4	2.33	
	1.7	3.1	13.5		0.7
	0.8	1.5	13.6		6.0
AVERAGE	1.4	2.7	16.8	2.9	3.4
STDV	0.6	0.8	2.2	1.0	3.8

Table 3.6 Results with over-dried dentin and formic acid



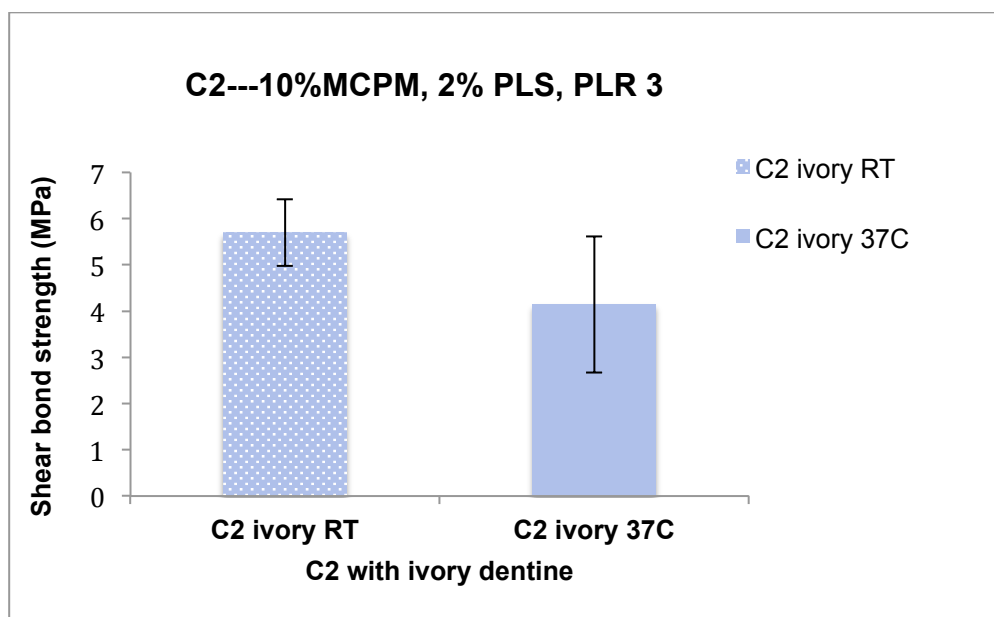
Graph 3.5 Graph presenting the shear bond strength results of Z250 with etch&i-bond, compared to A6/ C2 with form.acid and A6/ C2 with over-dried dentine

3.3.7 Tests with ivory dentine

The results with the ivory blocks are seen in the table below. The one column shows the shear bond strength in Mpa in room temperature, while the other column shows the results after 24 hours into the 37° incubator.

SBS Mpa	C2 ivory RT	C2 ivory 37°
	6.5	5.9
	5.3	5.3
	4.0	1.7
	5.6	2.1
	4.8	5.0
	4.5	7.4
	6.9	1.7
	4.3	3.9
	4.9	
	6.3	
	7.7	
	7.6	
Average	5.7	4.1
STDEV	0.8	2.1

Table 3.7 Shear bond strength results of C2 in ivory dentine in 37° temperature and in room temperature (23°)



Graph 3.6 Graph presenting the shear bond strength results using ivory dentine- C2 RT, C2 37°C incubator

The above results show that the temperature can have an effect on the shear bond strength of the C2 formulation when tested in ivory dentine, as the mean shear bond strength of the C2 in the blocks stored in the 37°C incubator is relatively lower than the mean shear bond strength when the formulations were tested in room temperature and there is also less consistency in the results.

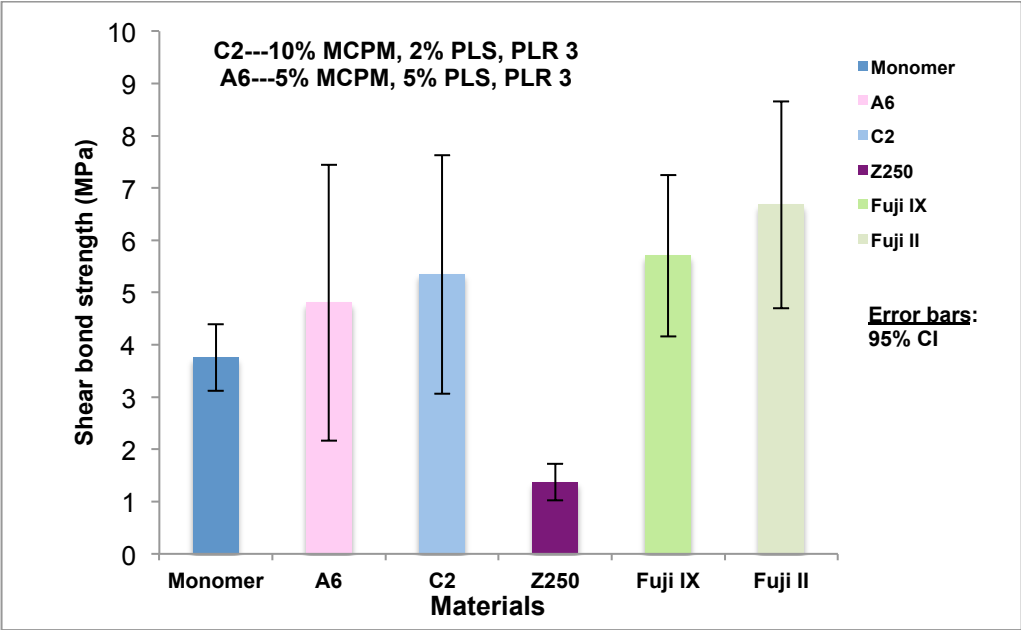
Overall, the C2 (10% MCPM, 2% PLS, PLR 3) and A6 (5% MCPM, 5% PLS, PLR 3) formulations have shown higher results compared to the Z250 when the shear bond strength was assessed without the use of etch and bond.

As seen in the Table 3.5, when etch and i-bond were used, the highest results were observed for the Z250 as well as for the A6 formulation. Using etch only resulted in the lowest results for the A6 formulation.

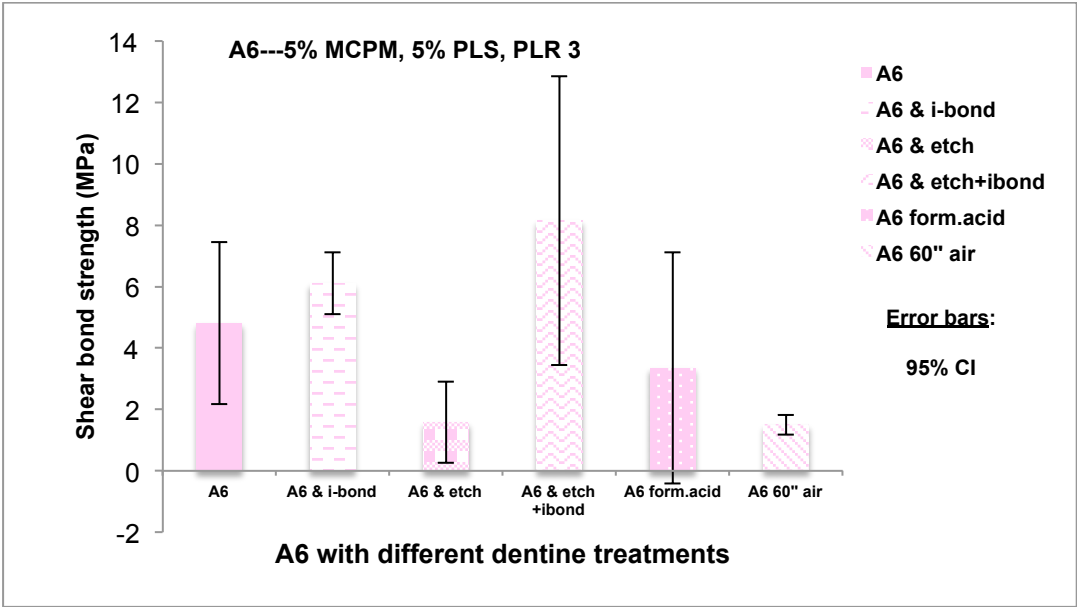
A summary of the results with the different treatments of the dentine surface for the two formulations is seen in the Graphs 3.7, 3.8 and 3.9. Graph 3.7 displays the results of the six different materials when tested without the use of etch and i-bond with Fuji II & IX having the highest shear bond strength followed by C2 and A6 respectively.

Graph 3.8 is a summary of all the different dentine treatments when the A6 formulation was assessed for the shear bond strength. A6 with etch & i-bond gave the

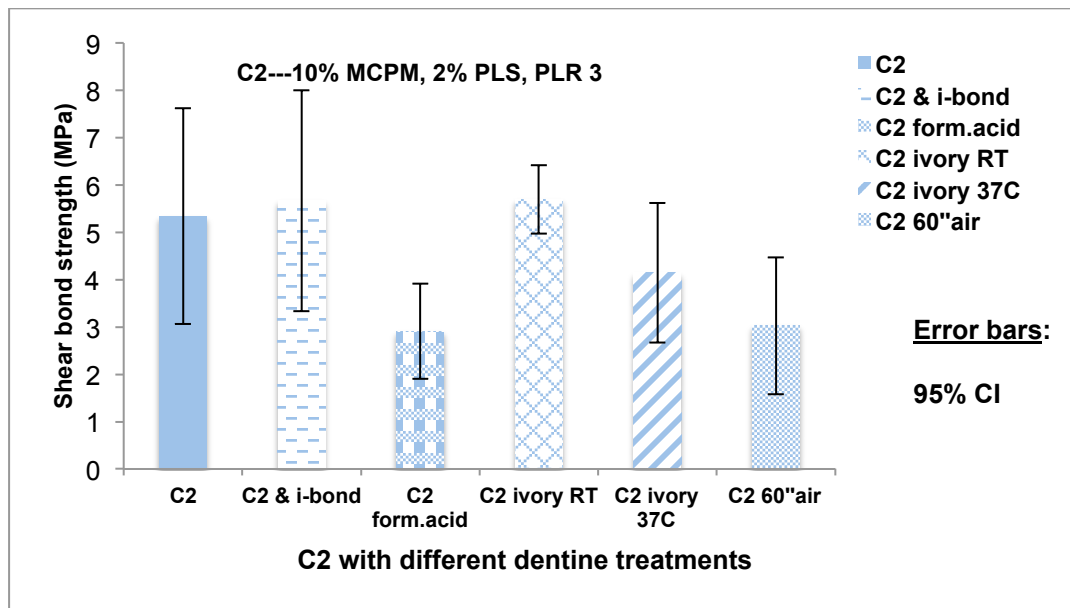
highest shear bond strength followed by the application of i-bond only, while etching of the dentine, over-drying and use of formic acid had a negative impact on the shear bond strength with very low results.



Graph 3.7 Summary of the results without etch & i-bond for the monomer, A6, C2, Z250, Fuji II-LC, Fuji IX without etch & i-bond



Graph 3.8 Summary of the shear bond strength results with A6 formulation using different combinations- A6 with i-bond, A6 with etch, A6 with etch & i-bond



Graph 3.9 Summary of SBS results for the C2 formulation without etch & i-bond, with i-bond only, formic acid, over-dried dentine and C2 with ivory dentine in room temperature and in 37°.

3.4 Statistical analysis of shear bond tests

The results of the shear bond strength for each material and each dentine pre-treatment were analysed for statistical significance. Levene's test was performed to assess the homogeneity of the results. When Levene's test shows significance, then a more generalised test should be selected for statistical analysis. In this case Levene test was performed and the result was $p < 0.05$, i.e. the data was not homogenous thus, a non-parametric test for independent samples was selected for the analysis (Kruskal- Wallis 1- Way ANOVA (k samples)).

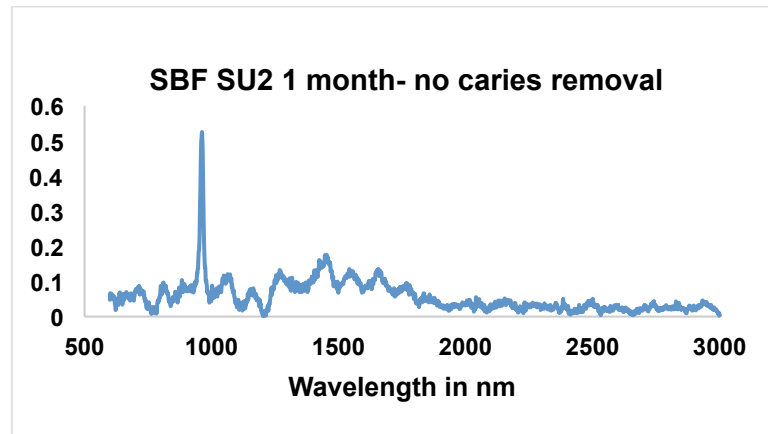
There was no statistically significant difference in the results of A6 when no etch and bond was used compared to the results with etch only or with bond only. The same was found for the C2 formulation as well, with no statistically significant difference in the shear bond strength between the groups with etch and/ or bond and without etch and bond.

Statistically significant difference was found between the shear bond strength of Z250 when both etch and bond were used compared to all the other materials and dentine treatments. The shear bond strength of the Z250 was significantly lower though when no etch and bond were used, compared to the shear bond strength of A6 or C2 when no etch and bond were used. No statistically significant difference was found between the SBS of A6 compared to C2 with all the different dentine treatments.

3.5 Results with RAMAN Spectroscopy

The RAMAN Spectroscopy was employed to assess the hydroxyapatite formation in the interface between the SU2 formulation and the infected dentine after 1 month in SBF.

The result is seen in the graph below, where hydroxyapatite was noted:



Graph 3.10 RAMAN Spectroscopy- SU2 no caries removal- 1 month SBF

3.6 Results with light microscope & SEM

The microscope and SEM (Scanning Electron Microscopy) were employed in order to assess the presence of resin tags in the interface between the new material (SU2) and the infected or affected dentine in the no caries removal or partial caries removal groups respectively. The results of the images are listed below:

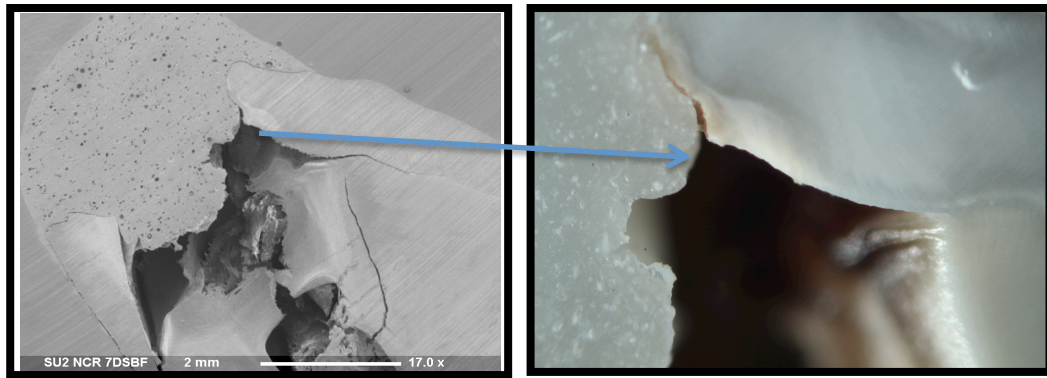


Figure 3.8, Figure 3.9 SEM image 2mm, 17.0x magnification of SU2 in no caries removal group. Microscope image of cavity filled with SU2, no caries removal group

Figures 3.8 and 3.9 show a Class II cavity filled with the SU2 formulation without caries removal and the image 3.8 has been taken with SEM in 2mm and 17.0x magnification. The figure shows the formation of a large gap where the infected dentine is, which is a result of the drying and collapse of the collagen fibrils of the carious dentine. On the other hand there is good adaptation in the infected and/or sound area of the dentine and enamel in the margins of the restoration. Figure 3.9 shows the same area of tooth/ material interface as pointed with the arrow, but the image is taken with the light microscope

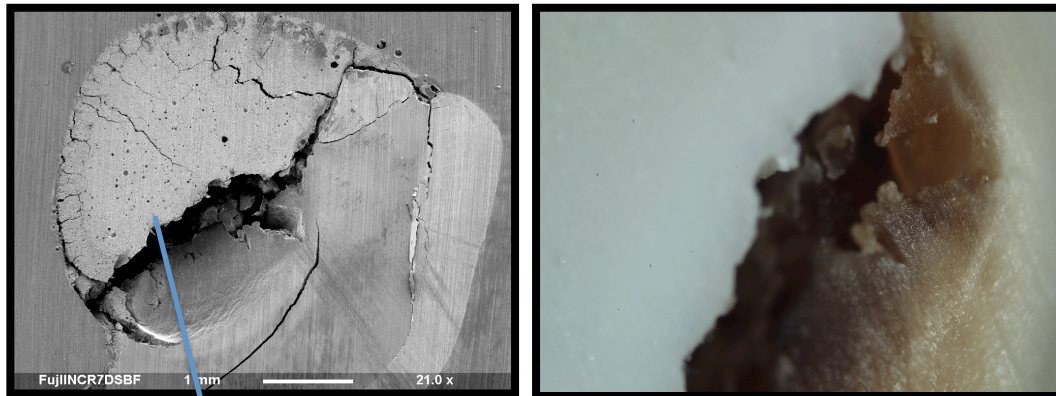


Figure 3.10, Figure 3.11 SEM image 1mm, 21.0x magnification of Fuji-II in no caries removal group. Microscope image of Fuji-II- carious dentine interface in the no caries removal group

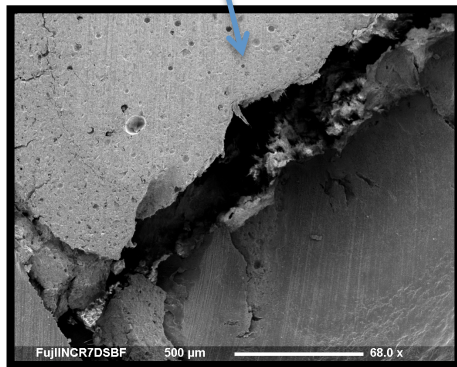


Figure 3.12 SEM image 500m, 68.8x magnification of Fuji-II- carious dentine interface in the no caries removal group

Figures 3.10, 3.11 and 3.12 show the Fuji-II in the no caries removal group. As we can see again the carious dentine seems to collapse as it dries during the SEM tests, causing the formation of a gap between tooth and material.

The figures 3.13 and 3.14 show the interface between the SU2 formulation and the affected dentine in the partial caries removal group. The first figure shows the microscope image of the interface, while the second figure shows an image of the same area taken with the SEM. The microscope is showing good adaption of the material into the cavity. The SEM image shows the presence of cracks in dentine and material, due to the fact that the sample gets dry due to the vacuum of the SEM.

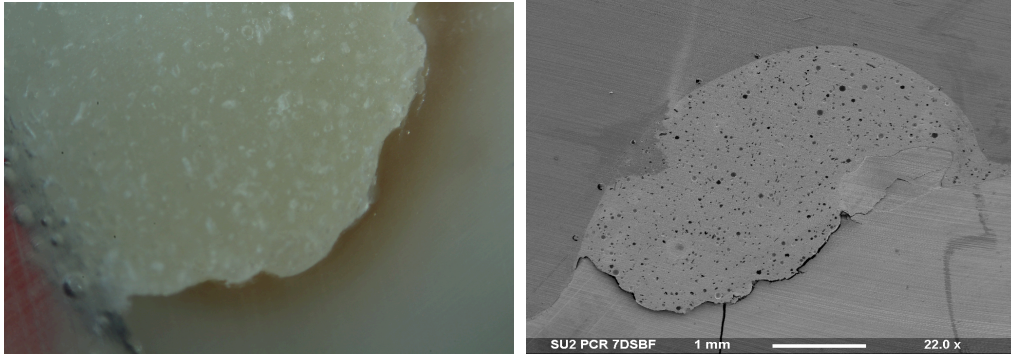


Figure 3.13, Figure 3.14 Microscope image of SU2 with partial caries removal and SEM image of SU2 with partial caries removal, 1mm, 22.0x magnification. Microscope shows good adaption of the material into the cavity. SEM shows the presence of cracks in in dentine, possibly due to the vacuum effect of SEM

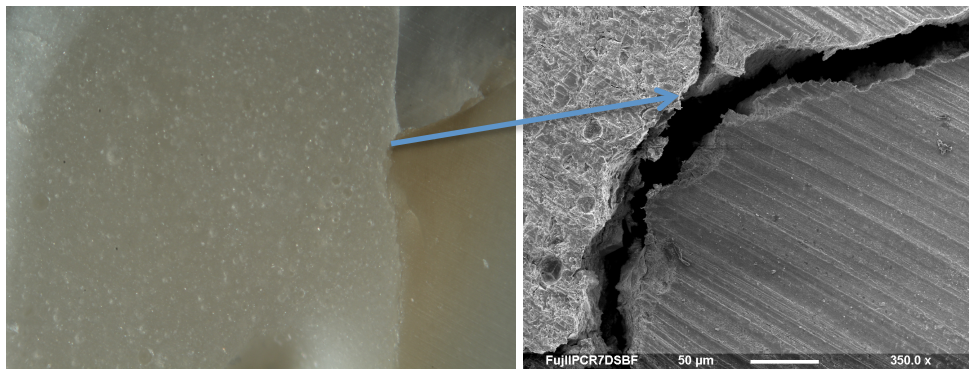


Figure 3.15, Figure 3.16 Microscope image Fuji II with partial caries removal and SEM image of Fuji II with partial caries removal, 50µm, 350.0x magnification. Microscope showing good adaption of the material into the cavity. SEM image with higher magnification shows no indication of resin tags

As seen in figures 3.14 & 3.15, the experimental formulation SU2 presents good adaption with the cavity and the affected dentine in the partial caries removal group. The SEM image shows some micro-gaps that can be because of the vacuum effect on the samples, which leads in drying of the dentine interface.

3.7 Results with Confocal Spectroscopy

The confocal microscopy revealed the presence of resin tags in the partial caries removal group. The green area in Figure 3.16 represents the naturally fluorescing carious dentine, while the red area in Figure 3.17 is from the rhodamine b dye and represents the composite SU2 penetrating the dentine.

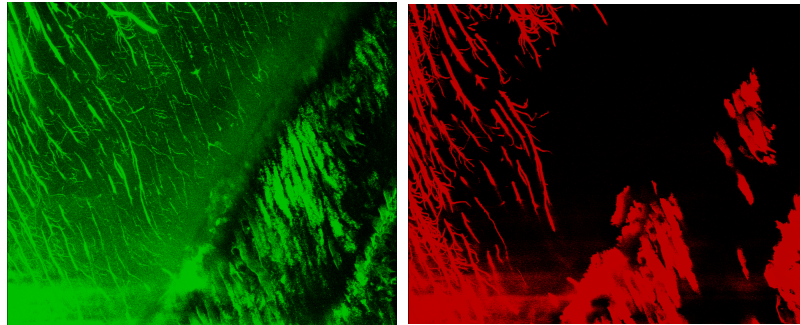


Figure 3.17, Figure 3.18 Confocal microscopy- resin tag formation up to 200 micron

For the experimental but not for the commercial material, resin tag formation up to 200 micron in length was observed in both highly infected dentine and the underlying disease affected dentine by the various microscopic techniques.

3.8 SEM- Resin tag formation

As seen in the figures 3.12a, b and c, resin tag formation was observed with the SEM, in 500.00x, 1200.00x and 1500.00x magnification.

Resin tag formation with the new composite suggests good sealing may be feasible without need for any adhesive.

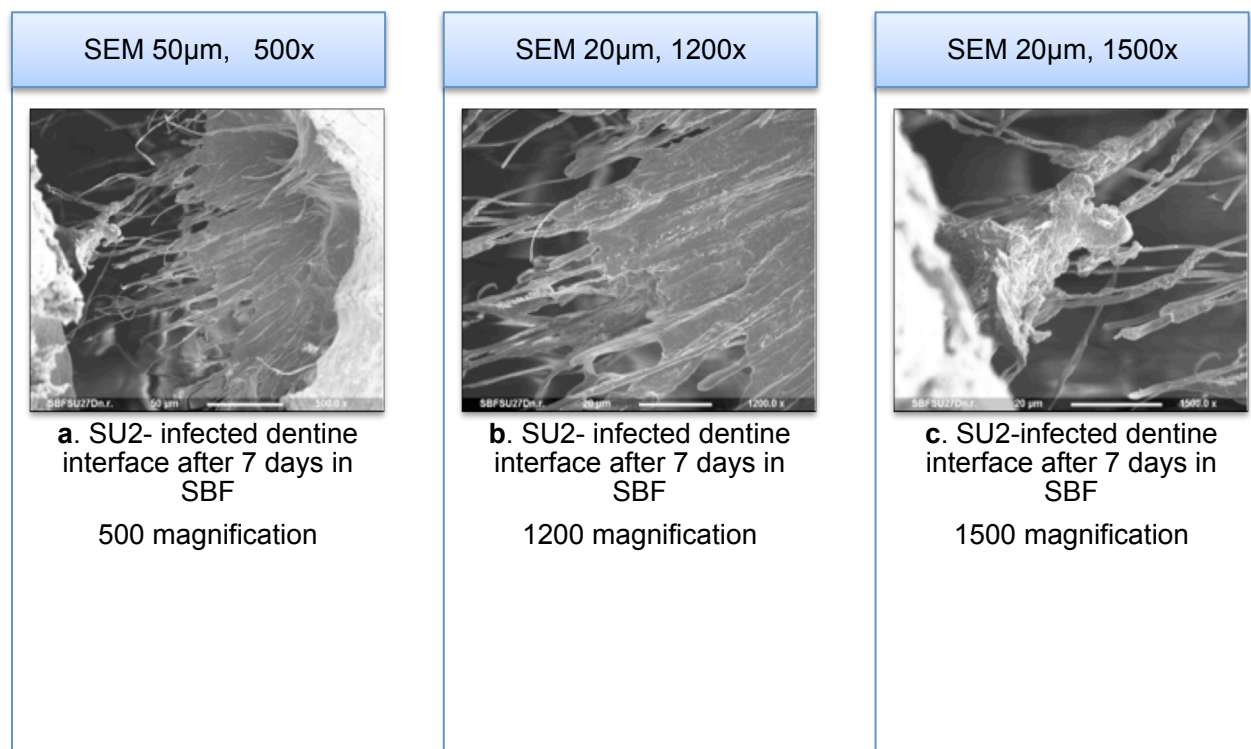


Figure 3.19a, 3.12b, 3.12c SEM images of the SU2-dentine interface, after 7 in days SBF and no caries removal in 500.0x, 1200.0x and 1500.0x magnification

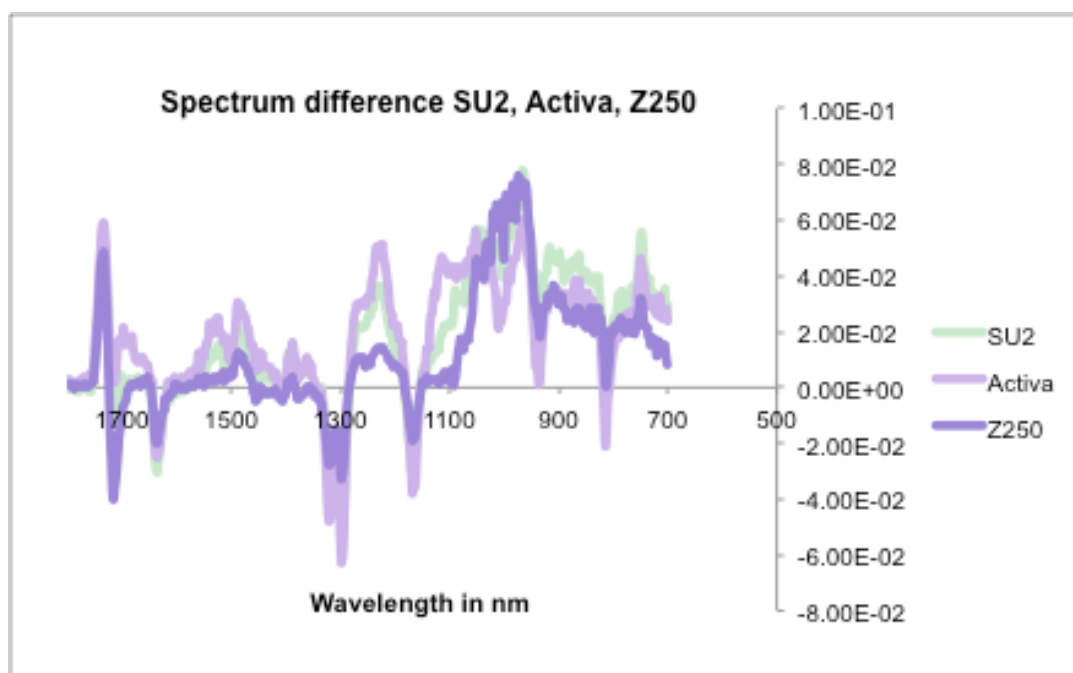
3.9 Results ATR FTIR: Monomer Conversion, Delay time, Reaction Rate, t 0.5

3.9.1 Chemistry of the materials

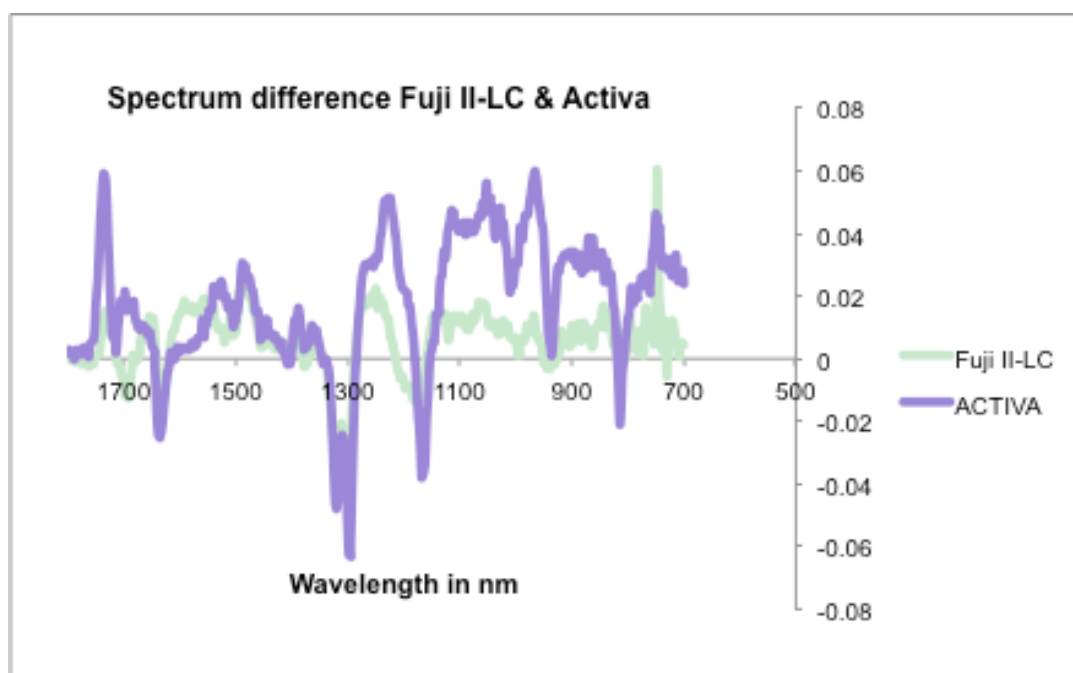
The Graphs 3.11 and 3.12 show the differences in reactions of the materials, due to the different chemistry of them. Graph 3.11 shows the comparison between the differences in spectrum of the SU2, Aactiva and Z250. SU2 and Aactiva both contain UDMA, thus the N – H group interacts with the C = O group around 1500nm wavelength. As a result, minor shifts in the polymerisation peaks of the materials can be seen.

Z250 has lower amount of methacrylate groups. In the area of 1300nm the level of reaction of the Z250 is lower, so less monomer is polymerizing. This results in lower shrinkage, but lower degree of conversion as well.

Graph 3.12 shows the comparison between Aactiva and Fuji II-LC. In Fuji II-LC we have twice the amount of methacrylates polymerizing compared to Aactiva. The O – H group is very water-soluble. Furthermore, Aactiva relies on water sorption to achieve reaction, while the RMGIC contains water in it's structure, thus it is able to have the acid- base reaction. The liquid phase of Fuji II- LC contains water and polyacrylic acid, resulting in quite low level of monomer. So the slight differences in the graphs can be explained by the different chemistries of the monomers.



Graph 3.10 Spectrum differences between the SU2, Activa and Fuji II-LC when tested in 2mm thickness and 20s curing time using ATR FTIR



Graph 3.11 Spectrum differences between Fuji II-LC and Activa when tested in 2mm thickness and 20s curing time using ATR FTIR

3.9.2 Results of Monomer Conversion assessment

The monomer conversion of the new formulation SU2 was assessed with the ATR FTIR.

For the experimental formulation (SU2) a mean of 77.1% monomer conversion was obtained at 2mm depth and 73.2% monomer conversion at 4mm depth. For the commercial Z250, the results were 58.7% for the 2mm and 54.7% in 4mm depth. Fuji II-LC had 88.1% for the 2mm and 91.9% in 4mm depth of cure. Finally, Activa had 58.5% MC in 2mm thickness.

The following tables and graphs show the results of the monomer conversion, the delay time and the reaction rate as well as the $t_{0.5}$ of the four different materials SU2, Fuji II-LC, Activa and Z250 in 2mm thickness and curing time 20 seconds and in 4mm thickness and curing time 40 seconds.

3.9.3 2mm thickness – 20 seconds curing time

The tables 3.8, 3.9, 3.10, 3.11 and the graphs 3.11, 3.12, 3.13, 3.14 display the mean monomer conversions, delay times, reaction rates and $t_{0.5}$ respectively for each of the four materials tested with ATR FTIR.

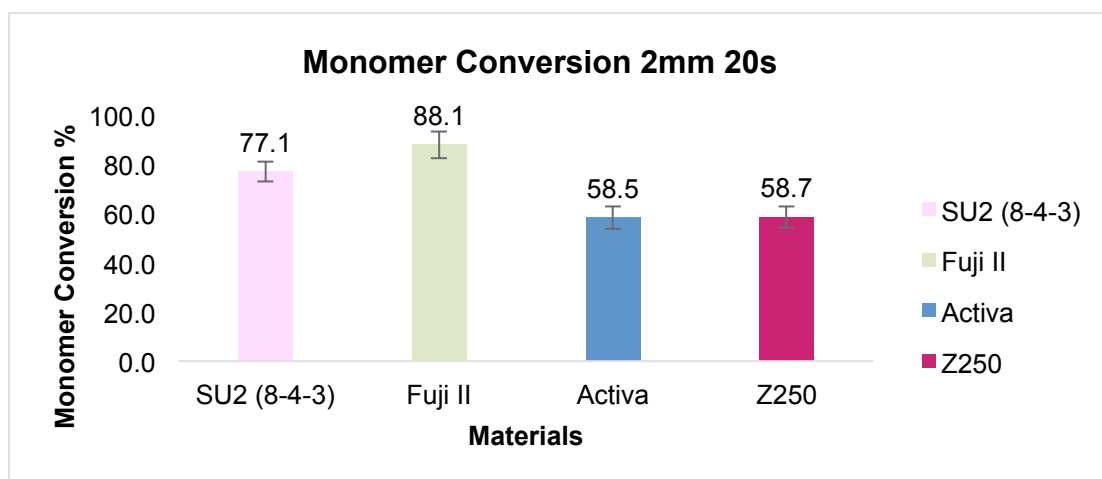
As seen in table 3.8, the SU2 formulation had mean monomer conversion 77.1%, followed by Activa with 58.5% and Z250 with 58.7%. The Fuji II had 88.1% monomer conversion, relatively higher compared to the rest as expected.

The delay time was 7.5 sec for the SU2, followed by Fuji II, Activa and Z250 with 6 sec, 4.8 sec and 4.2 sec delay times respectively.

3.9.3.1 Monomer Conversion – 2mm 20s

Monomer Conversion (%)	2mm thickness			
	SU2 (8-4-3)	Fuji II	Activa	Z250
Mean STDV	81.8	81.8	56.1	59.5
	74.7	91.5	55.5	62.5
	74.9	91.0	63.8	54.1
	77.1	88.1	58.5	58.7
	4.0	5.5	4.6	4.3

Table 3.8 Results of ATR FTIR for the monomer conversion in 2mm thickness- SU2, Fuji II, Activa and Z250



Graph 3.12 Monomer Conversion of SU2, Fuji II-LC, Activa and Z250 in 2mm thickness and curing time 20s

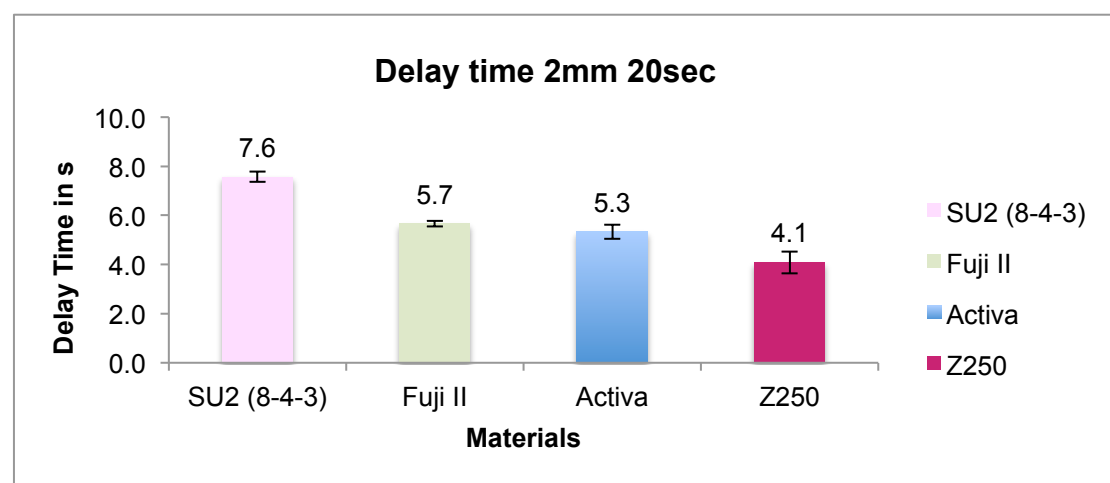
The results for the delay time (time before any reaction of the material) obtained from the ATR FTIR can be seen in table 3.9 and in Graph 3.12 for the 2mm thickness and three repetitions for each material. A mean of 7.5 s

3.9.3.2 Delay time - 2mm 20s

Table 3.9 and Graph 3.14 present the results of the delay time in 2mm thickness and 20s curing for the four different materials. SU2 had mean delay time 7.5s, followed by Fuji II-LC with 6s, while Aactiva and Z250 had the smallest delay time with 4.8s and 4.2s respectively.

2mm thickness				
Delay time (s)	SU2 (8-4-3)	Fuji II	Activa	Z250
Mean STDV	5.8	5.8	5.8	4.6
	7.4	5.6	5.2	2.4
	9.2	6.6	3.5	5.5
	7.5	6.0	4.8	4.2
	1.7	0.5	1.2	1.6

Table 3.9 Results of the delay time in s for the SU2, Fuji II-LC, Activa and Z250



Graph 3.13 Delay times in sec for the SU2, Fuji II-LC, Activa and Z250 in 2mm thickness and 20sec curing time

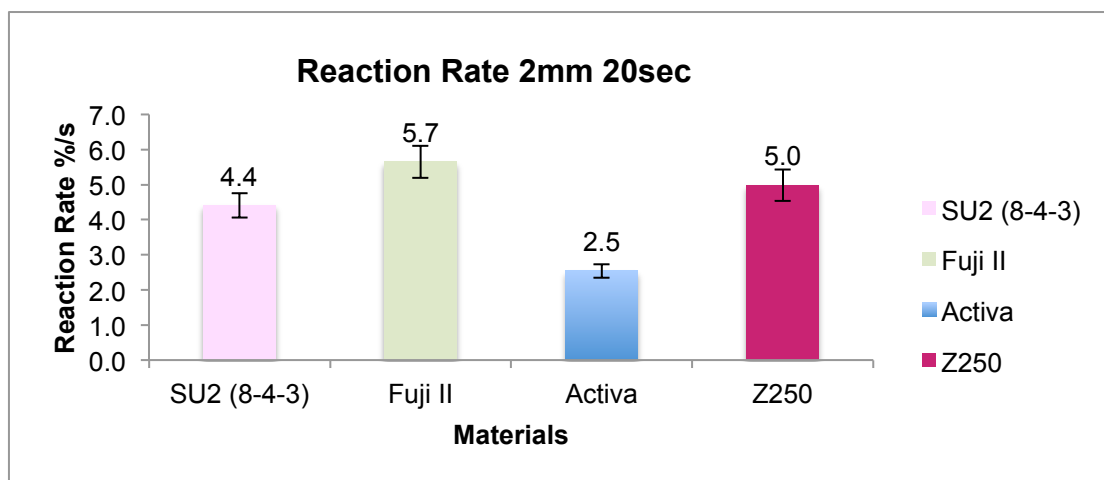
The mean of the delay time as seen in Graph 3.14 was 7.6s for SU2, for Fuji II –LC was 5.7s, followed by Activa with 5.3 and Z250 had the lower delay time with mean 4.1s.

3.9.3.3 Reaction Rate – 2mm 20s

Reaction Rate (%/s)	2 mm thickness			
	SU2 (8-4-3)	Fuji II	Activa	Z250
	4.3	4.3	4.3	5.3
	4.1	5.7	2.3	3.8
	5.0	7.1	2.4	5.2
Mean	4.5	5.7	3.0	4.8
STDV	0.5	1.4	1.1	0.8

Table 3.10 Results obtained from the ATR FTIR for the reaction rate in 2mm thickness, 20sec of curing for SU2, Fuji II, Activa and Z250

The reaction rates of the four materials in 2mm thickness were 4.5 %/s for the SU2, while the Activa presented 3 %/s and Fuji II and Z250 had 5.7 and 4.8 %/s respectively. The results are presented in Graphs

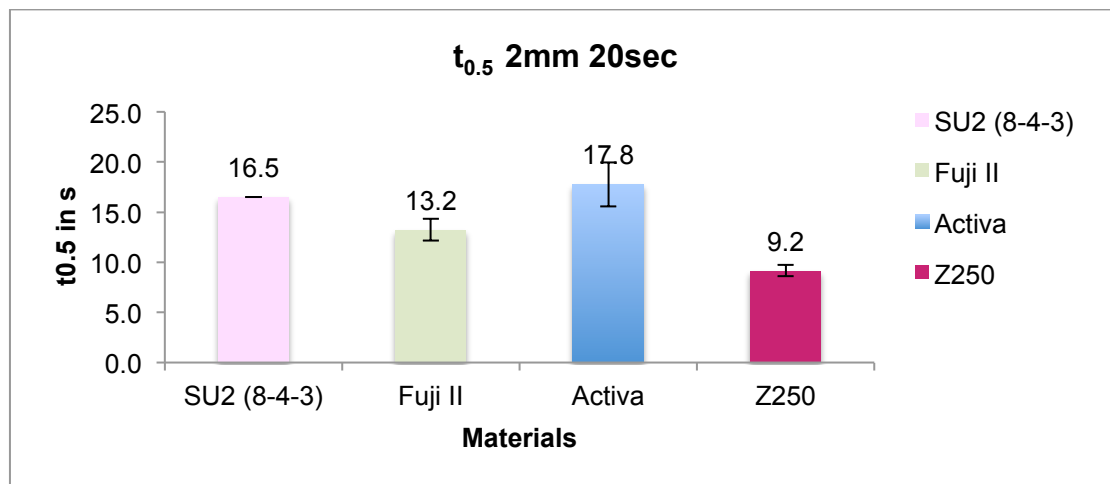


Graph 3.14 Reaction rate in %/s for SU2, Fuji II-LC, Activa and Z250 in 2mm thickness and 20sec curing time

3.9.3.4 $T_{0.5}$ – 2mm 20s

$t_{0.5}$	2mm thickness			
Mean STDV	SU2 (8-4-3)	Fuji II	Activa	Z250
	15.4	15.4	15.4	10.3
	16.5	13.7	17.2	10.7
	16.7	13.1	17.0	10.7
	16.2	14.1	16.5	10.6
	0.7	1.2	1.0	0.2

Table 3.11 Results of $t_{0.5}$ for the 2mm thickness, 20sec curing time for SU2, Fuji II, Activa & Z250



Graph 3.15 $t_{0.5}$ in sec for the SU2, Fuji II-LC, Activa and Z250 in 2mm thickness and 20sec curing time

3.9.4 4mm thickness – 40 seconds curing time

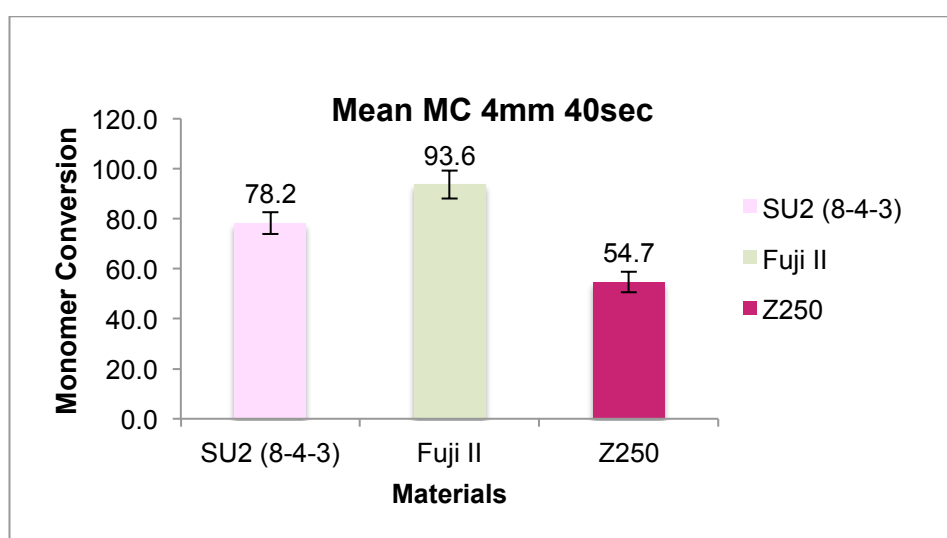
3.9.4.1 Monomer Conversion

The same materials were tested in 4mm thickness, doing 3 repetitions for each material and the results of the mean monomer conversion can be seen in Table 3.12 and Graph 3.15.

The SU2 presented with 73.2% mean monomer conversion, which is higher than that of the Z250 that had 54.7%, but lower compared to Fuji II that had 92.9 %.

Monomer Conversion %	4mm thickness		
	SU2 (8-4-3)	Fuji II	Z250
	81.3	91.0	52.3
	65.1	89.3	52.3
		98.4	59.4
Mean	73.2	92.9	54.7
STDV	11.5	13.1	4.1

Table 3.12 Monomer conversion in 4mm thickness and 40sec curing time for SU2, Fuji II, Activa and Z250

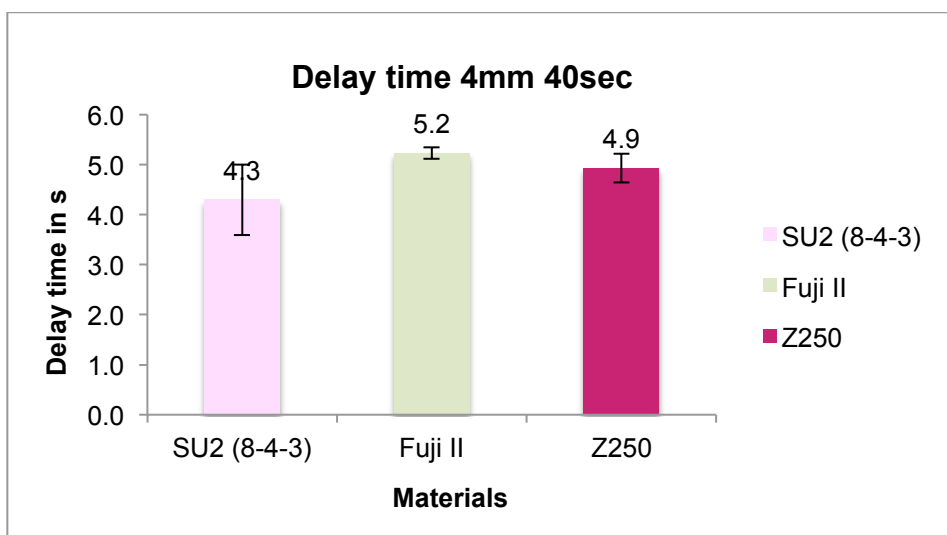


Graph 3.16 Monomer Conversion in % obtained from the ATR FTIR for the SU2, Fuji II-LC, Activa and Z250 in 4mm thickness and 40sec curing time

3.9.4.2 Delay time 4mm- 40sec

Delay Time (sec)	4mm thickness		
	SU2 (8-4-3)	Fuji II	Z250
	5.8	8.0	2.7
	8.5	8.5	7.0
		9.2	4.9
Mean	7.2	8.6	4.9
STDV	1.9	5.2	2.2

Table 3.13 Delay time obtained from the ATR FTIR for SU2, Fuji II & Z250 in 4mm thickness and 40sec curing time



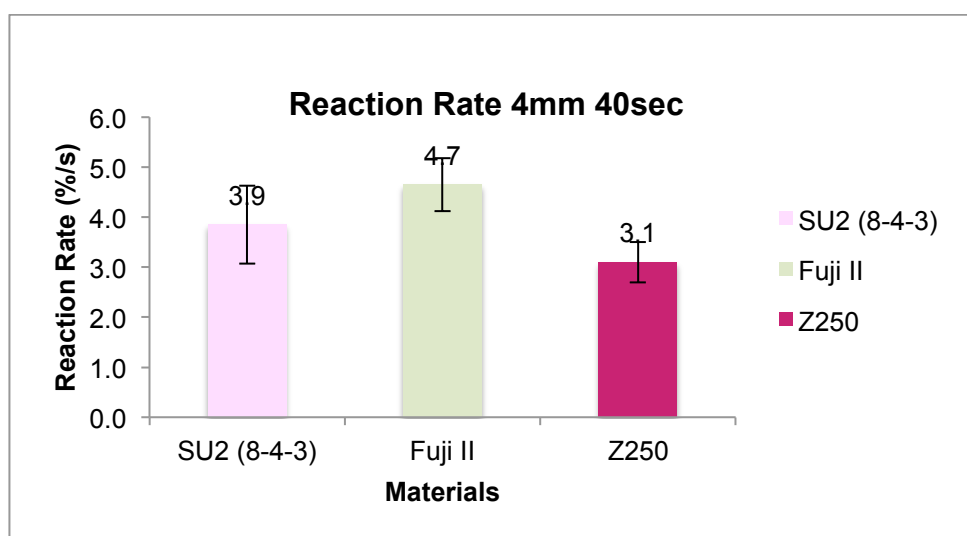
Graph 3.17 Delay time in sec for the SU2, Fuji II-LC and Z250 in 4mm thickness and 40sec curing time

3.9.4.3 Reaction Rate- 4mm 40sec

Table 3.14 and graph 3.17 present the reaction rate of the materials in 4mm thickness.

Reaction Rate (%/sec)	4mm thickness		
	SU2 (8-4-3)	Fuji II	Z250
	3.1	3.5	0.7
	2.7	2.7	2.1
		5.0	3.1
Mean	2.9	3.7	2.0
STDV	0.3	1.2	1.2

Table 3.14 Delay time obtained from the ATR FTIR for SU2, Fuji II, Activa & Z250 in 4mm thickness and 40sec curing time



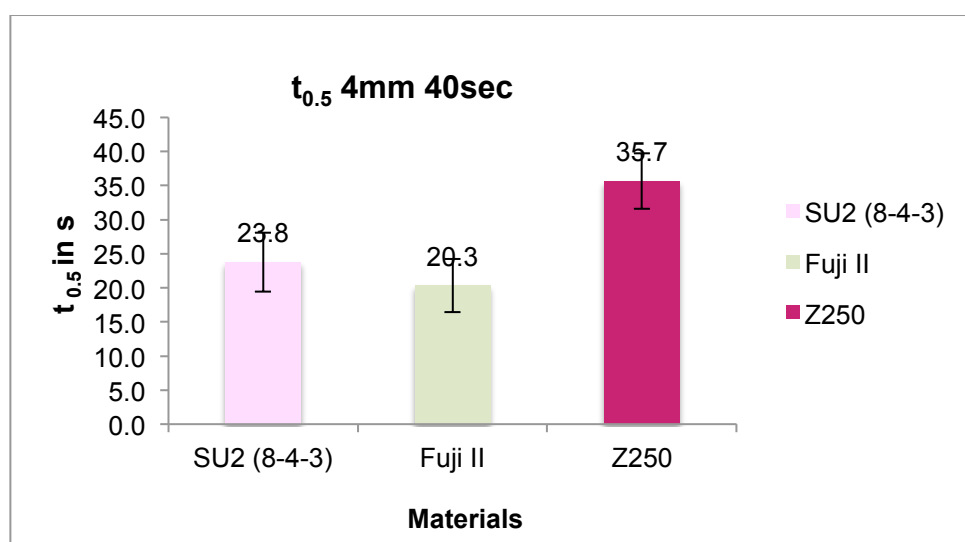
Graph 3.18 Reaction Rate in %/s for the SU2, Fuji II-LC, Activa and Z250 in 4mm thickness and 40sec curing time

3.9.4.4 $T_{0.5}$ - 4mm 40sec

Table 3.15 and Graph 3.18 present the results of the time needed in order to achieve half of the monomer conversion ($t_{0.5}$).

$t_{0.5}$ (sec)	4mm thickness		
	SU2 (8-4-3)	Fuji II	Z250
	26.8	20.3	14.0
	20.7	16.4	20.3
		24.2	12.7
Mean	23.8	20.3	15.7
STDV	4.3	3.9	4.1

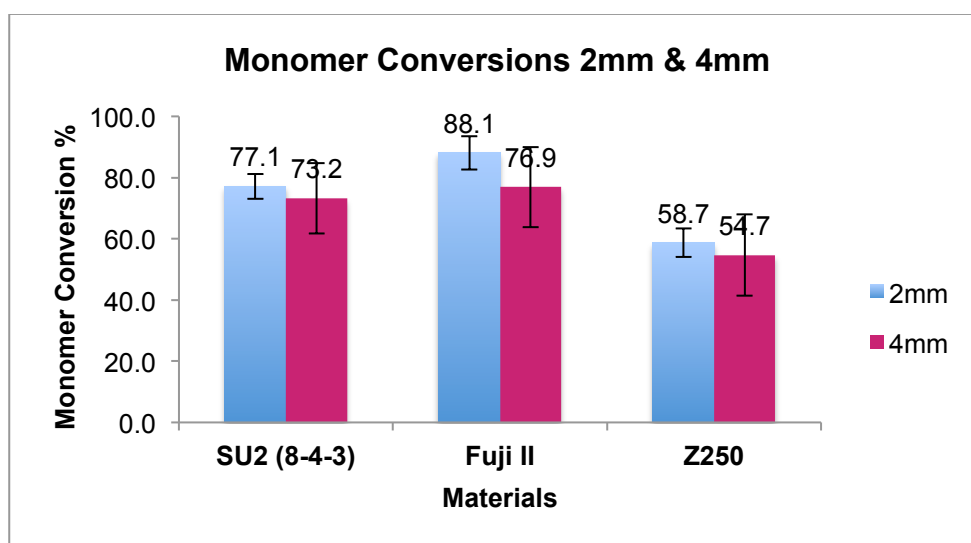
Table 3.15 $t_{0.5}$ obtained from the ATR FTIR for SU2, Fuji II and Z250 in 4mm thickness and 40sec curing time



Graph 3.19 $t_{0.5}$ in sec for the SU2, Fuji II-LC and Z250 in 4mm thickness and 40sec curing time

3.10 Monomer Conversions 2mm & 4mm comparison

Graph 3.19 shows the comparison of the monomer conversions of the materials in the two different thicknesses. Fuji II- LC had the highest monomer conversion in both 2mm and 4mm thickness, 88.1% and 76.9% respectively, followed by the SU2 with 77.1% and 73.2% MC in 2mm and 4mm thickness. Lowest MC was for Z250, which presented 58.7% and 54.7% MC for 2mm and 4mm respectively.



Graph 3.20 Graph showing the comparison of the monomer conversions for the four materials (SU2, Fuji II, Activa & Z250) in 2mm and 4mm thickness

3.11 Statistical analysis of FTIR results

The results of the degree of monomer conversion were analysed for statistically significant differences using the SPSS and performing a one- way analysis of variance (ANOVA). Levene's test was initially performed to assess the homogeneity of the results. Levene test did not show significance ($p>0.05$), i.e. the data was homogenous, thus a parametric test was employed for the analysis (1- Way ANOVA). The degree of monomer conversion of the four materials in 2mm thickness was analysed and found that the SU2 experimental composite and the Fuji II-LC had significantly higher degree of conversion compared to both Z250 and Aactiva, whereas no statistically significant difference between the monomer conversions of SU2 and Fuji II-LC was found. The results of the statistical analysis are presented in the Appendix. The results on the 4mm thickness were omitted from the statistical analysis as not enough results could be obtained due to limitations of ATR FTIR and materials' shrinkage.

Chapter 4

4 Discussion & Conclusions

4.1 Summary of the results

This project has been dealing with the shear bond strength assessment, resin tag formation and monomer conversion of new experimental composite formulations described by the acronym SMART. The teeth that were used for the shear bond strength tests were sound molars and premolars according to the ISO guidelines on testing of the adhesion to tooth structure. Literature has shown that most of the research on the adhesion of dental materials is done using sound dentine (SoD) (Isolan et al, 2018), however, especially with the recent changes in caries management approach (Innes, 2017), the most common substrate for bonding is the caries affected dentine (CAD) (Isolan et al, 2018).

The aim of the new formulations assessed in this project was the application without prior etching and bonding of the dentine, hence, shear bond tests were done following this approach and the results were compared to those of commercial materials (Fuji II, Fuji IX and Z250) and to those after various different treatments of the dentine surface.

For the assessment of resin tag formation, carious primary molars were employed. These teeth were filled either with the experimental SU2 material or with the commercial Fuji II- LC.

4.2 Shear Bond Strength

A major factor to be taken into consideration when assessing the intraoral performance of a composite material is bonding. The shear bond strength is a measure of the adhesion of a material to the tooth structure. It is important to assess the shear bond strength as it determines how much force is necessary in order to break the bond between material and tooth and it can be a predictor of the performance of the material when forces are applied in the oral cavity.

The initial outcomes of the shear bond strength presented with great variation, high standard error and very low shear bond strength results for all the different formulations that were tested apart from Fuji II & Fuji IX, which had mean shear

bond strength of 6.8 Mpa and 5.7 Mpa respectively. Fuji II- LC sets initially through resin polymerization and Fuji IX via an acid-base reaction creating a chemical bond to the dentine. As a result, these materials don't need the prior application of etch or bond. However, literature has shown that Fuji II and Fuji IX lack strength and wear off with time, so they cannot be used as permanent restorations in areas of the mouth that the forces are increased (Xie et al, 2000).

The storage medium of teeth was also reviewed during this project. The initial protocol included disinfection of the teeth in thymol solution, which was subsequently changed to Chloramine T 1%. Studies have shown that both these storage media have a minimal effect on the dentine and on the bond strength (Mobarak et al, 2010; Haller et al, 1993).

Another factor that can explain the low initial shear bond strength results is the difficulty to overcome some limitations of the human tooth, which are thoroughly describe in the next part. These include the presence of dentinal tubules and fluid and the presence of pulp. In this project some of the human teeth used for shear bond tests would not keep any material in place. When the tests were repeated using a tooth ID and the same tooth was used for each material, better consistency was obtained in the results, while specific teeth could have higher or lower shear bond strength measurements for all the different materials tested, coming in agreement with the previous observations and giving some indication that the results can be tooth- related.

The use of etch only appeared to have a negative impact on the bond strength and the formulation had hardly any bond into the tooth surface for all the teeth that were used apart from one that showed 5.34 MPa shear bond strength. This can be explained by the fact that A6 has self-etching properties. As a result, further etching could lead to the breakdown of the dentine collagen fibrils and would not enhance the composite penetration. This finding comes in agreement with studies testing the bond strength and micro-leakage of a commercial self-adhesive (Vertise Flow, Kerr) (Munck et al, 2004; Rengo et al, 2012). However, one study testing the same self-adhesive material has shown beneficial effect of pre-etching the dentin with phosphoric acid (Leung et al, 2005; Poitevin et al, 2013). According to a more recent systematic review, it seems that etching in universal adhesives has a positive impact on the enamel and improves the bond strength, but this is not the case for dentine (Rosa et al, 2015).

The formic acid pre-treatment of the dentine had a negative impact on the shear bond strength in this project. This could be because of the collapse of collagen fibrils due to the formic acid, leading to material inability to penetrate the dentine. A recent study though that was assessing one-step self-etch adhesives found that the prior conditioning of the dentine with NaOCl can deproteinize the dentine smear layer and lead to a significant improvement in the bond strength of all adhesives tested (Thanatvarakorn et al, 2018).

The over-drying of the dentine (60s air) had a negative effect on the shear bond strength when the A6 and C2 formulations were tested. This result could be explained by the hydrophilic nature of the two experimental formulations, which need some moisture in order to have reaction. On the other hand, the Z250 with dry and etched dentin followed by the bonding agent gave results that were significantly higher as Z250 has hydrophobic nature, thus over-drying the dentine was positive for the shear bond strength. On the contrary, the experimental composites are hydrophilic materials and presence of moisture could benefit the bond strength.

The ivory dentine resulted in more consistent outcomes of the shear bond strength, something that would be expected, given the fact that it comes as a single bulk, without differences in density of the dentinal tubules and without the presence of dentinal fluid and pulp as it happens with human dentine. The ivory dentine was tested in two different temperatures and the results showed that the temperature could affect the shear bond strength of the C2 formulation when tested in ivory dentine. The mean shear bond strength for the C2 in the ivory blocks kept in the 37°C incubator appears to be lower compared to the mean shear bond strength when the formulations were tested in room temperature and the 37° group also presented less consistency in the results.

Overall, the C2 (10% MCPM, 2% PLS, PLR 3) and A6 (5% MCPM, 5% PLS, PLR 3) formulations have shown higher results compared to the Z250 when the shear bond strength was assessed without the use of etch and bond. Specifically, a statistically significant difference was found between the results of A6 without etch and bond and those of Z250 without etch and bond with the latter being significantly lower. However, these results are not yet conclusive, as further research is being done in order -first of all- to create a standardised protocol of testing the shear bond strength in human dentine and be able to achieve repeatable results, given the fact that the human dentine has some limitations.

4.3 Limitations of the shear bond tests in human dentine

For the tests that have been done during this project in order to assess the shear bond strength of the new novel composite formulations, human dentin was used.

This methodology though, has some limitations.

First of all, sound human teeth are difficult to obtain, as ethical approval and consent from the patients is necessary. As a result, the sample size for these tests is small and the outcomes need to be carefully considered before being generated. Also, human teeth are small in size something that adds one more challenge in the technique of shear bond strength tests. For these tests each tooth used for testing was polished to remove the remaining of the previous material. The change in the level of dentine tested every time and the proximity to the pulp could have an additional impact on the shear bond strength outcomes obtained, due to the changes in density of the dentinal tubules. Furthermore, when the pulp was reached after the polishing, these teeth had to be discarded and this increased the need for a larger sample size of human teeth.

In addition to the above, in order to do the shear bond tests, the teeth were embedded into resin blocks and a flat dentin surface was used for the application of the material. This application though is not a realistic representation of what we face when we apply the materials in the clinical setting, where there is most of the times a cavity that can add some mechanical retention as well. As a matter of fact, the mechanical retention of the material is not taken into consideration during these shear bond tests.

Another aspect is the composition of human dentin. Human dentin consists of the dentinal tubules, which contain fluid and their size and density can vary the more we go near the pulp. Therefore, every time a tooth is polished with the 500 grit disc for the several test repetitions, that can have an effect on the result of the next test, as we move closer to the pulp. Even when the teeth are initially cut, there is always a small difference in the levels of the dentine exposed in each tooth, which can have impact on the shear bond strength of the material tested. Not only human dentine has limitations though. Ivory dentine, although being similar to human dentine, it lacks the pulp and dentinal tubule fluid, as a result it cannot mimic the clinical setting in the oral cavity sufficiently.

Finally, there is difficulty in achieving the appropriate moisture control, so that the experimental composite formulations will have the optimal bond strength. These new materials are hydrophilic, so a completely dry dentin surface can have a negative impact on the shear bond strength, as seen from the results with the over-dried dentine. However, it is important to establish the ideal amount of moisture in the dentine that could give the best possible bonding with the material.

4.4 Resin tag formation

One of the main mechanisms of bonding between adhesive composite materials and dentine is the resin tag formation. The mechanisms of bonding to enamel and dentine are driven by their superficial demineralisation through the etching, followed by the infiltration of the resin monomers and creation of the resin tags. Self-etch adhesives contain acidic monomers that act as primers and condition the dentine in order to promote the so-called “hybridization” of the dentine without the need of prior etch application. Hybridisation is the infiltration of dentine collagen fibrils with the resin. (Van Meerbeek et al, 2011).

In this project carious primary molars were used with minimal or partial caries removal in order to assess the formation of resin tags. The material used (SU2 formulation) has self-adhesive properties due to the presence of 4-META and MCPM. It was found that resin tags could be created in both infected dentine as determined with SEM and affected dentine as seen with the confocal microscopy suggesting adequate self-sealing ability.

4.5 Monomer Conversion

In this project, the final formulation of the experimental composites SU2 was assessed in order to determine the monomer conversion and compare it with a commercial GIC (Fuji II- LC), a commercial composite (Z250, 3M) and a commercial compomer (Activa).

The cytotoxicity of dental composite materials has been attributed to the release of residual monomers, a result of an incomplete polymerization reaction, or to the by-products of resin matrix degradation processes. The mechanisms of cytotoxicity are related to the short-term release of free monomers occurring during the monomer–polymer conversion (Goldberg et al, 2008). It has been shown that well cured resin composites are less cytotoxic than those with lower degree of conversion.

The degree of conversion of a monomer can be measured in order to determine its mechanical properties as well as its biocompatibility (Demarco et al, 2013). Higher degree of conversion means that the material has better mechanical properties and less contents leaking out of it following the polymerisation process (Ilie et al, 2014), a quality that results to better biocompatibility of the material. In this project the ATR FTIR was employed in order to determine the monomer conversion of the new formulation (SU2) in % and compare it with the monomer conversions of commercial materials such as Z250, Fuji II and Activa. The experimental composite material was found to have a high monomer conversion with a mean of about 73% and 77.1% in 2mm and 4mm depth of cure respectively, suggesting lower release of free monomers, thus better biocompatibility compared to the commercial Z250. It was also found that the degree of MC was significantly higher compared to that of Z250.

The composite formulation tested in this project contained UDMA a component that has been shown to improve monomer conversion when used as a bulk monomer (Liaqat et al, 2015). The SU2 displayed higher conversion compared to the Z250 composite in both 2mm and 4mm thickness. The monomer conversion was also higher compared to Activa in the 2mm thickness. This can be explained by the presence of PPGDMA as a diluent monomer, which is a large and flexible molecule containing fewer carbon- carbon double bonds (Walters et al, 2016).

Overall the experimental composite formulation SU2 had better monomer conversion than a commercial composite at both 2 mm and 4 mm depth suggesting that it may

be placed in a single step without layering. It can also show that SU2 formulation has lower amount of non-polymerized monomer, thus less toxicity and better biocompatibility. The RMGIC had much higher levels of conversion but this is expected due to the presence of mono instead of dimethacrylate monomers.

In the limitations of the ATR FTIR tests are included the inconsistent results of the monomer conversions when the materials were tested in 4mm thickness. In the 4mm depth of cure, some results (mainly for the SU2 and for Activa) were not consistent or could not be obtained at all, due to the sample shrinkage that resulted in losing contact with the FTIR diamond even after multiple repetitions.

4.6 Conclusions

To sum up, in this project three experimental formulations were assessed for the shear bond strength, resin tag formation and monomer conversion. The C2 (10% MCPM, 2% PLS) experimental formulation was found to have higher bond strength compared to the A6 (5% MCPM, 5% PLS) when no etch or i-bond were used, the difference however, was not found statistically significant. The commercial Z250 had always the lowest bond strength when used without etch and bond, however it was still best performing when etch and bond were used together, prior to the application of the material and that difference was statistically significant compared to the materials placed without etch and bond.

The SU2 composite (8% MCPM, 4% PLS) had better monomer conversion than a commercial composite at both 2 mm and 4 mm depth suggesting it may be placed in a single step without layering. The RMGIC had much higher levels of conversion but this is required due to the presence of mono instead of dimethacrylate monomers. Resin tag formation with the new composite was observed with SEM and suggests that good sealing may be feasible without need for any adhesive.

4.7 Research for the future

Further research is necessary in order to maintain a standardised protocol of repeatable tests of the shear bond strength in human dentine. This test has been one of the most common tests used for the assessment of materials' adhesion to tooth structure, but its validity is often being questioned due to the aforementioned limitations. The results that have been presented so far are not yet conclusive as this is an ongoing research and more tests will be necessary before coming to a definite conclusion. Future research will be necessary to include more shear bond strength tests, in order to achieve the ideal moisture level of the dentine for better results with the new formulations. It is also necessary to increase the sample size of the teeth in order to be able to generate the findings and draw conclusions. New tests will need to be added in the research, such as microleakage and micro-tensile bond strength.

Regarding the resin tag formation, it is necessary to research further in order to assess how much caries needs to be removed and what is the ideal moisture level of the cavity from the dentine in order to achieve the best outcome.

The limitations of the ATR FTIR need to be further investigated as in the 4mm thickness some samples were losing contact with the ATR diamond thus no results could be obtained even after multiple repetitions.

Chapter 5

5 References

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Chapter 6

6 Appendix

6.1 ATR FTIR – Statistical Analysis of Monomer Conversions

One – Way Analysis of Variance (ANOVA) for the analysis of the results of monomer conversions in 2mm thickness and 20s curing time for the SU2, Z250, Activa and Fuji II-LC.

```
GET DATA /TYPE=XLSX
/FILE='E:\Stats ANOVA FTIR.xlsx'
/SHEET=name 'Sheet1'
/CELLRANGE=full
/READNAMES=on
/ASSUMEDSTRWIDTH=32767.
EXECUTE.
DATASET NAME DataSet1 WINDOW=FRONT.
ONEWAY monomerconversion BY Group
/STATISTICS HOMOGENEITY
/MISSING ANALYSIS
/POSTHOC=TUKEY T3 ALPHA(0.05).
```

Oneway

Notes

Output Created	01-AUG-2018 21:10:37
Comments	
Input	Active Dataset DataSet1
	Filter <none>
	Weight <none>
	Split File <none>
	N of Rows in Working Data 12
	File
Missing Value Handling	Definition of Missing User-defined missing values are treated as missing.
	Cases Used Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax	ONEWAY monomerconversion BY Group /STATISTICS HOMOGENEITY /MISSING ANALYSIS /POSTHOC=TUKEY T3 ALPHA(0.05).
Resources	Processor Time 00:00:00.06
	Elapsed Time 00:00:00.20

Test of Homogeneity of Variances

monomer conversion

Levene Statistic	df1	df2	Sig.
.279	3	8	.839

ANOVA

monomer conversion

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1912.273	3	637.424	29.632	.000
Within Groups	172.092	8	21.511		
Total	2084.365	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: monomer conversion

			Mean Difference	Std. Error	Sig.	95% Confidence Interval
			(I-J)			Lower Bound
Tukey HSD	1.0	2.0	-.2337	3.7869	1.000	-12.361
		3.0	-29.6300*	3.7869	.000	-41.757
		4.0	-18.6503*	3.7869	.005	-30.777
	2.0	1.0	.2337	3.7869	1.000	-11.893
		3.0	-29.3963*	3.7869	.000	-41.523
		4.0	-18.4167*	3.7869	.005	-30.544
	3.0	1.0	29.6300*	3.7869	.000	17.503
		2.0	29.3963*	3.7869	.000	17.269
		4.0	10.9797	3.7869	.077	-1.147
	4.0	1.0	18.6503*	3.7869	.005	6.523
		2.0	18.4167*	3.7869	.005	6.290
		3.0	-10.9797	3.7869	.077	-23.107
Dunnett T3	1.0	2.0	-.2337	3.6406	1.000	-16.180
		3.0	-29.6300*	4.1359	.009	-47.953
		4.0	-18.6503*	3.5503	.028	-34.299
	2.0	1.0	.2337	3.6406	1.000	-15.713

	3.0	-29.3963*	4.0096	.010	-47.450
	4.0	-18.4167*	3.4024	.024	-33.297
3.0	1.0	29.6300*	4.1359	.009	11.307
	2.0	29.3963*	4.0096	.010	11.343
	4.0	10.9797	3.9279	.200	-6.972
4.0	1.0	18.6503*	3.5503	.028	3.002
	2.0	18.4167*	3.4024	.024	3.536
	3.0	-10.9797	3.9279	.200	-28.931

Multiple Comparisons

Dependent Variable: monomer conversion

			95% Confidence Interval
	(I) Group	(J) Group	Upper Bound
Tukey HSD	1.0	2.0	11.893
		3.0	-17.503
		4.0	-6.523
	2.0	1.0	12.361
		3.0	-17.269
		4.0	-6.290
	3.0	1.0	41.757
		2.0	41.523
		4.0	23.107
	4.0	1.0	30.777
		2.0	30.544
		3.0	1.147
Dunnett T3	1.0	2.0	15.713
		3.0	-11.307
		4.0	-3.002
	2.0	1.0	16.180
		3.0	-11.343
		4.0	-3.536
	3.0	1.0	47.953
		2.0	47.450
		4.0	28.931
	4.0	1.0	34.299
		2.0	33.297
		3.0	6.972

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

monomer conversion				
Group		N	Subset for alpha = 0.05	
			1	2
Tukey HSD ^a	1.0	3	58.467	
	2.0	3	58.700	
	4.0	3		
	3.0	3		
	Sig.		1.000	.077

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

6.2 Statistical analysis of Shear Bond Strength

The results of the Kruskal-Wallis non-parametric test are displayed in Figure 6.1 and in Table 6.1 when the A6 and C2 formulations were compared with Z250, Fuji II and Fuji IX and in Figure 6.2 and Table 6.2 when the A6 formulation results were compared to the shear bond strength of Z250, Fuji II-LC and Fuji IX.

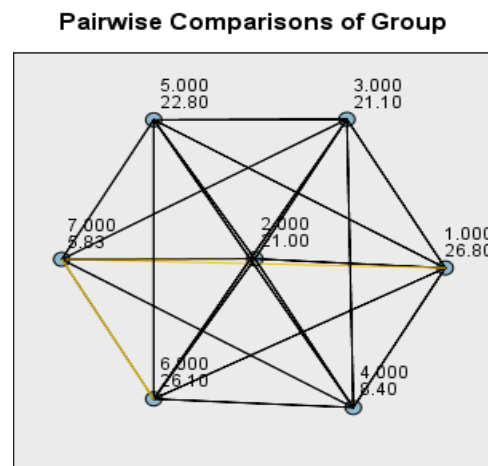


Figure 6.1 Graph displaying the pairwise comparisons following Kruskal- Wallis tests

Each node shows the sample average rank of Group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
7.000-4.000	2.567	6.378	.402	.687	1.000
7.000-2.000	15.167	6.378	2.378	.017	.366
7.000-3.000	15.267	6.378	2.394	.017	.350
7.000-5.000	16.967	6.378	2.660	.008	.164
7.000-6.000	20.267	6.378	3.178	.001	.031
7.000-1.000	20.967	6.378	3.287	.001	.021
4.000-2.000	12.600	6.662	1.891	.059	1.000
4.000-3.000	12.700	6.662	1.906	.057	1.000
4.000-5.000	-14.400	6.662	-2.162	.031	.644
4.000-6.000	-17.700	6.662	-2.657	.008	.166
4.000-1.000	18.400	6.662	2.762	.006	.121
2.000-3.000	-.100	6.662	-.015	.988	1.000
2.000-5.000	-1.800	6.662	-.270	.787	1.000
2.000-6.000	-5.100	6.662	-.766	.444	1.000
2.000-1.000	5.800	6.662	.871	.384	1.000
3.000-5.000	-1.700	6.662	-.255	.799	1.000
3.000-6.000	-5.000	6.662	-.751	.453	1.000
3.000-1.000	5.700	6.662	.856	.392	1.000
5.000-6.000	-3.300	6.662	-.495	.620	1.000
5.000-1.000	4.000	6.662	.600	.548	1.000
6.000-1.000	.700	6.662	.105	.916	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

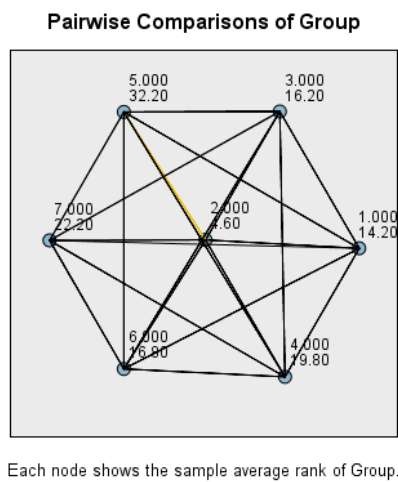


Figure 6.2 Graph of the pairwise comparison of Kruskal-Wallis test for the

Each node shows the sample average rank of Group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
2.000-1.000	9.600	6.481	1.481	.139	1.000
2.000-3.000	-11.600	6.481	-1.790	.073	1.000
2.000-6.000	-12.200	6.481	-1.883	.060	1.000
2.000-4.000	-15.200	6.481	-2.345	.019	.399
2.000-7.000	-17.600	6.481	-2.716	.007	.139
2.000-5.000	-27.600	6.481	-4.259	.000	.000
1.000-3.000	-2.000	6.481	-.309	.758	1.000
1.000-6.000	-2.600	6.481	-.401	.688	1.000
1.000-4.000	-5.600	6.481	-.864	.388	1.000
1.000-7.000	-8.000	6.481	-1.234	.217	1.000
1.000-5.000	-18.000	6.481	-2.777	.005	.115
3.000-6.000	-.600	6.481	-.093	.926	1.000
3.000-4.000	-3.600	6.481	-.555	.579	1.000
3.000-7.000	-6.000	6.481	-.926	.355	1.000
3.000-5.000	-16.000	6.481	-2.469	.014	.285
6.000-4.000	3.000	6.481	.463	.643	1.000
6.000-7.000	-5.400	6.481	-.833	.405	1.000
6.000-5.000	15.400	6.481	2.376	.017	.367
4.000-7.000	-2.400	6.481	-.370	.711	1.000
4.000-5.000	-12.400	6.481	-1.913	.056	1.000
7.000-5.000	10.000	6.481	1.543	.123	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

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